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EFFECTS OF CERTAIN ORGANIC CARRIERS AND METAL CHELATES ON GROWTH OF CORN IN NUTRIENT CULTURE

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BY

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES (CONTINUED)

ABSTRACT

This dissertation deals with the use of certain organic carriers, including metal chelates, in plant nutrient culture. There are presented a series of nine experiments with corn grown under greenhouse conditions. It has been divided into four parts:

Part I. This is concerned with the substitution of an organic form of phosphorus for potassium dihydrogen phosphate, the usual source. The results show that even at high pH (6.0-7.0) ethyl ammonium phosphate is a good substitute for potassium dihydrogen phosphate; and, unlike inorganic phosphates, it does not make iron unavailable to plants by forming precipitates with ferric chloride.

Part II. This deals with the substitution of calcium acetate for calcium nitrate which is commonly used as a source of calcium in culture solutions. It is found that calcium acetate not only is a good source of calcium but also buffers the nutrient solution to maintain it at a fairly high and consistent pH level. Furthermore, calcium acetate appears to stimulate root growth to a greater extent than calcium nitrate.

Part III. Ethylene diamine tetraacetic acid (EDTA) in low concentrations (10 micromoles and below) is found to be distinctly beneficial to the growth of corn, particularly to its root development, in solution culture. It has been suggested that this beneficial effect may be due to a growth-

promoting property of EDTA in low concentrations.

Corn plants growing in nutrient solution are adversely affected by excess of copper sulfate (0.4 micromole and higher). EDTA can prevent copper toxicity in corn up to a certain level. The biochemical basis for the modifying effect of EDTA on copper toxicity has been discussed in detail.

A similar experiment with manganese indicates that EDTA fails to prevent chlorosis in corn caused by excess manganese in the nutrient solution.

Part IV. The upper non-toxic level for "Versenol" (N-hydroxy ethyl ethylene diamine triacetic acid) in nutrient solution is found to be 20 micromoles per liter. Unlike EDTA, "Versenol" in low dilutions does not seem to have any particular beneficial effect on the root growth of corn.

"Versenol" magnesium chelate, when supplied in non-toxic concentrations (6 micromoles) and with proper adjustment of the composition of the nutrient solution, can substitute effectively for magnesium sulfate as a source of magnesium. "Versenol" calcium chelate, however, proves to be a poor substitute for calcium acetate as a source of calcium.

INTRODUCTION

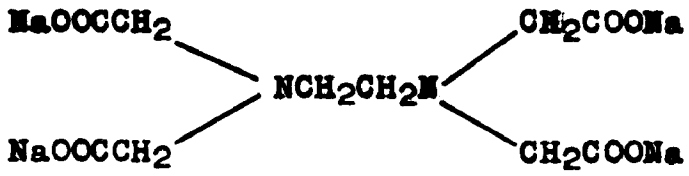
In recent years a new field of study has been opened to plant physiologists with the discovery of the synthetic chelating agents which form very stable complexes with heavy metals such as iron, copper, manganese, and zinc.

Martell and Calvin (51) have recently published an extensive account of the chemistry of metal chelate compounds. The word "chelate" comes from the Greek word "kelos" meaning "claw" and refers to the ring configuration that results when a metal combines with two or more donor groups of a single molecule. Metals bound in chelate rings lose their cationic characteristics and become a part of a complex anion. Naturally occurring organic chelates may be illustrated by chlorophyll in which magnesium is chelated in pyrrole rings, and by haemoglobin which has iron in the nucleus of its molecule.

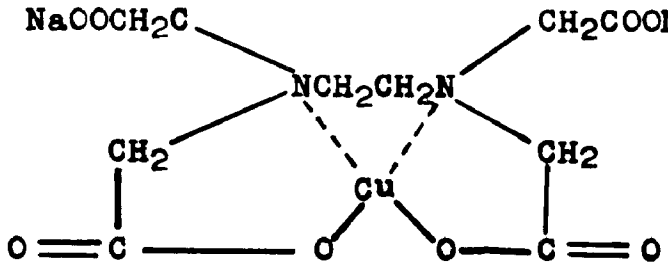
The best known of all the synthetic amino-polycarbolxylic acids that form water-soluble metal chelates is ethylene diamine tetraacetic acid, hereafter referred to as EDTA. This compound forms five-atom strain-free chelate rings, and there are six atoms--two of nitrogen and four of oxygen--which can donate electrons to metals. The structures of the sodium salt of EDTA and its metal complexes appear in Fig. 1.

The binding of EDTA with metals is stronger for some metals than for others. In a scale descending from

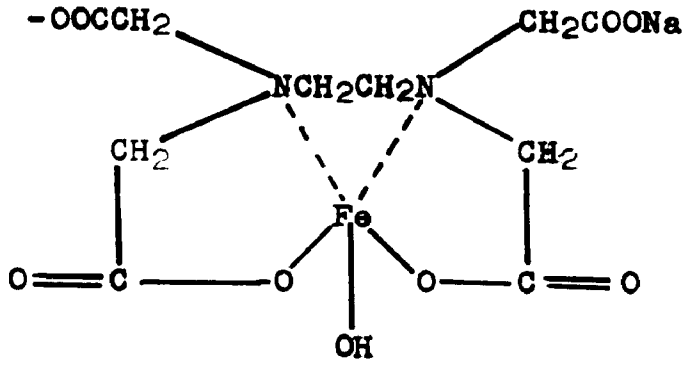
Fig. 1 Structure of EDTA
and its metal complexes.



Structure of Na4EDTA



Structure of Na4EDTA
upon chelation of a divalent metal (copper)



Structure of Na4EDTA
upon chelation with trivalent metal (iron)

strong to weak chelates, the order is ferric ion, copper, zinc, ferrous ion, manganese, calcium, and magnesium. Hydrolysis of these metal chelates generally occurs at high pH values; for example, at levels of pH higher than 6.0 the OH^- competes with EDTA for Fe^{+++}, and at about pH 8.0 the OH^- concentration is sufficiently high to remove iron effectively from EDTA. Likewise, at the other end of the scale at approximately pH 2.0, the chelate becomes ionized to the extent that virtually none of the metal will be complexed. In general, iron and copper are chelated most effectively in acid solutions, whereas calcium and magnesium are chelated more strongly in alkaline solutions (73).

There are a number of other synthetic chelating agents that are in use: diethylene triamine pentaacetic acid ("chel 330"), N-hydroxy ethyl ethylene diamine tri-acetic acid ("Versenol"), cyclohexane trans 1, 2-diamino tetraacetic acid ("chel 600").

The recent pioneer work of Jacobson (38) and Stewart and Leonard (72) in the use of Fe-EDTA as a source of iron for plants has opened up new research possibilities in the field of plant nutrition with relation to metal chelates. Since then a considerable amount of literature has been published concerning the use of metal chelates in plant nutrition. Chelated zinc, manganese, and calcium also have been tried in both soil application and foliar spray. Most of the work done with metal chelates, however, relates to soil application; and very little is known about the role of various metal chelates in solution culture.

The results obtained in the soil applications depended not only on the metal chelates themselves, "but often more, on the composition of the soil, the method of application, the plant species..." (73). The author believes that in solution culture, factors such as hydrogen-ion concentration, essential macro- and micro-elements and their interactions can be more conveniently controlled and studied than in soil. The following quotation from Arnon (2) would emphasize this point:

> The ubiquitous complexity of heterogeneous soil components often complicates the interpretation of observed plant responses. The difficulty arises in attempting to distinguish between a direct and an indirect effect of a given soil treatment on the plant...It is with artificial nutrient media, water and sand cultures, that the essential status of the various elements found in plants and derived from the soil, was established...The artificial culture continues to be a powerful and discriminating tool in evaluating the indispensability of inorganic nutrients in plant nutrition.

In view of the fact that very little is known about the use of organic carriers--metal chelates particularly--in nutrient culture, the author presents the results of a series of investigations which may be of considerable fundamental interest. For the sake of clarity of presentation the entire dissertation will be divided into four parts which are interrelated: (I) Sources of iron and phosphorus and their interactions; (II) Ca-acetate as a source of calcium and its advantages; (III) Experiments on EDTA and its metal complexes; (IV) Experiments on "Versenol" and its metal complexes.

REVIEW OF LITERATURE

A proper balance of different nutrient elements has been considered a fundamental requirement in plant nutrition by various authors (63, 64, 39, 68, 66, 25, 55, 6, 12, 13, 60, 76, 77). This is true for plants grown in solution as well as those grown in soil.

Biddulph (6), Rediske and Biddulph (60), and DeKock (19) emphasized the desirability of an optimum Fe : P ratio for the growth of plants in nutrient culture. "The relative amounts of Fe and P absorbed by the plant determine whether the plant will show chlorotic symptoms or appear healthy" (19). Recently Brown (11) has made an extensive review of literature on iron chlorosis. It has been suggested that iron absorption by plants is considerably reduced by excess phosphorus--particularly at pH of about 6.0 or higher (25). In a series of papers, Marsh (47, 48, 49, 50) discussed the comparative values of various inorganic and organic sources of iron in solution culture. Reed and Haas (61) studied the effect of various organic compounds on the solubility of iron in nutrient solutions. Hopkins and Wann (37) successfully used iron citrate as a source of iron for Chlorella growing in solution culture.

There is no report in the literature of the use of organic forms of phosphorus in solution culture. Various organic phosphates, however, have been used as fertilizers

in soil, and many of them have been found to be as available to plants as inorganic phosphates (69, 70). It should be of considerable fundamental interest to determine the use of organic phosphates in nutrient culture in relation to iron supply.

Hydrogen-ion concentration of the nutrient solution has an important bearing on the availability of certain nutrient elements. Guest and Chapman (27), however, did not find any appreciable direct effect of pH on citrus seedlings growing in sand and solution culture unless the pH-values were as extreme as 2.0 or 11.0. Michael (53) found that uptake of magnesium and calcium from culture solutions by young corn and rye plants was considerably lower at pH 4.0 than at pH 6.0 or 7.5; whereas the uptake of potassium and phosphorus was not affected by low pH. Smith (65) studied the growth of citrus seedlings with nitrate and ammonium forms of nitrogen in solution cultures; and he observed a significant reduction in growth -- irrespective of the form of nitrogen--at pH 4.0 as compared to pH 6.0. He also reported that this reduction in growth was associated with a reduction of K^{+}, Ca^{++}, and Mg^{++} concentration in the foliage. Steinberg (71) showed that complete availability of iron, magnesium, and calcium to plants in nutrient solution was possible in both neutral and acid media if the content of the culture jar was stirred vigorously semi-weekly, insuring adequate contact between root and precipitate. Wadleigh et al (76) suggested that

an equilibrium exists between iron-stability, pH of tissue fluid, and phosphate-ion concentration of plant sap. Wadleigh and Shive (77) showed that the effect of the kind of nitrogen--ammonium or nitrate--on the uptake of cations by corn plants was of much greater importance than the pH of the culture solution.

Nightingale (56) has shown that as a result of differential absorption and respiration, rapid changes in pH occur in the solution immediately surrounding the roots. This warrants the use of suitable buffers in nutrient solution to keep the pH of the solution consistent throughout an experiment. Calcium acetate may be one of these buffers, and it may play the dual roles of a buffer and a source of calcium. There is no reference in the literature to such use of calcium acetate in the solution culture.

EDTA has been used extensively and effectively in such industries as textile, soap, detergent, rubber, cosmetic, germicide, and pulp-paper. Ca-EDTA often is used in treating heavy metal poisoning. The potentialities of metal chelates in plant nutrition have not been realized until recently. Stewart and Leonard (73), Haertl and Martell (28), and Wallace (78) have made excellent reviews on this subject. In search of a more satisfactory means of keeping iron from precipitating in nutrient solutions, Jacobson (38) grew plants in various molar ratios of iron and the potassium salt of EDTA. Tomato, sunflower, corn, and barley grew well with 5 to 25 ppm Fe supplied as the chelate. Injury occurred to both corn and tomato when

the nutrient solution contained 50 or 100 ppm chelated iron. Stewart and Leonard (73) found 5 ppm Fe-EDTA to be very suitable for citrus growing in sand culture. Heck and Bailey (31) used oxine, carbamate, and salicylic acid as chelating agents. They concluded that it was not feasible to chelate heavy metals in nutrient solutions because there was a point below which the concentration was not effective and a point above which the concentration was injurious to the plants. Waris (85) suggested the significance of chelating agents for growing algae in nutrient solution. Rasmussen (59) reported that Fe-EDTA was an effective source of iron in solution culture as long as the pH was kept below 7.0. Iron chlorosis which developed because of high alkalinity of nutrient solution could be corrected by addition of acid or "Versenol" iron chelate. He also concluded that "microelemental chelates may be added to nutrient solutions in somewhat higher concentration than the corresponding salts without the microelements becoming toxic to the plants".

It is interesting to note that "the use of chelates other than those of iron has not been studied widely in nutrient cultures" (73). Since much difficulty arises in supplying an inorganic form of iron to plants growing in nutrient cultures, research workers have emphasized iron and not bothered to study the availability of other metal complexes. In soil application, however, the use of iron as well as certain other metal chelates has been extensively studied.

Atkinson and Wright (3) reported that chelate applications to the soil could change the soil composition considerably. They have shown that leaching of calcareous soil with a chelating agent (EDTA) can result in the mobilization, transport, and redeposition of iron and aluminum and the development of a profile with well-defined horizons. Holmes and Brown (35) reported that the same chelate behaved differently depending on the kind of soil. In Florida nearly half the citrus groves have been found deficient in iron. In these acid soils Fe-EDTA has been very effective in alleviating the deficiency (43, 75). The problem has been much more difficult in calcareous soils. The iron chelate of hydroxy-EDTA (Fe-EDTA-OH) was found to be considerably more effective than Fe-EDTA in correcting lime-induced chlorosis (44, 16). Wallace and North (80) reported correction of lime-induced chlorosis in avocado by Fe-EDTA. Wallace et al (81) made comparative studies of a number of different chelating agents in relation to their supplying micronutrients to woody plants in alkaline and calcareous soils. In another publication (82) they expressed the opinion that the iron-salt of diethyl triamine pentacetic acid--hereafter referred to as DTPA--was more effective than Fe-EDTA in correcting chlorosis. Kroll (41) suggested that ferric chelate of "chel 138", an aromatic phenolic analogue of EDTA was a very effective iron chelate in correcting lime-induced chlorosis. Malcolm (46) reported that the most successful chelating agent for the correction

of iron chlorosis in alkaline soil was "Versenol"--N-hydroxy ethyl ethylene diamine triacetic acid. A number of other workers have attempted to determine the behavior of different iron chelates in various types of soil (42, 79, 35). Bould (8, 9, 10), in a series of publications, has indicated the effective use of iron chelates in controlling lime-induced chlorosis, both in foliar spray and in soil application.

One of the other metal chelates studied extensively is zinc chelate (1, 5, 15, 75). Soil application of Zn-EDTA and "Versenol" zinc chelate corrected the zinc deficiency in peach and sweet cherry, but the spray of zinc chelate was in no case a satisfactory corrective for zinc deficiency (5). In contrast with this observation, Stewart and Leonard (75) found that the only dependable method of controlling zinc deficiency in citrus was by foliar spray of zinc chelate. Butler and Bray (15) observed that the type of soil determined whether or not extra addition of Zn-EDTA would be effective. Alben (1) reported some preliminary results of treating rosetted pecan trees with chelated zinc.

A number of workers (90, 91, 92) have made interesting observations on the use of metal chelates in correcting chlorosis in ornamentals.

Behavior of iron chelates in plants often depends on the other constituents in the growth medium. Reuther and Smith (62) suggested that excessive accumulation of copper in the soil is the major reason for iron chlorosis and that

low pH of the soil greatly aggravates the toxicity of copper by increasing its availability. Other experimental results (34) show that the presence of phosphate ions in the clay and concentration of calcium ions in solution will affect the behavior of iron chelates. It is believed (54) that when a plant is deficient in iron, calcium is usually abnormally low in leaves, while K^{+}, Cu^{++}, Zn^{++}, and Mn^{++} may--one or all--be abnormally high. Under these conditions iron chelate can bring the nutrient level to a normal balance. Stewart and Leonard (72) associated iron deficiency not only with excess of Cu^{++}, Mn^{++}, Co^{++}, and Ni^{++} ions but also with a lack of potassium. Westgate (89) reported that chelated iron, either as a foliar spray or as a soil amendment, corrected iron chlorosis in various vegetables and ornamentals, even in presence of high copper concentration.

Until recently, it was debatable whether metal chelates as such could be absorbed by plants. Extensive studies by Stewart and Leonard (74) with chelates separately tagged with Fe^{55}, Fe^{59}, Zn^{65}, and Cl^{4} have shown that the metals in chelated form are much more available to plants than their inorganic salts. Wallace and North (80) have found that N^{15} is taken up by plants receiving N^{15}EDTA. This could mean that the entire chelate is being absorbed or that the plant is taking up the degradation product of the ligand. More recent work by Weinstein (87) and DeKock (20) suggests that the entire chelate is absorbed by plants. Perkins and Purvis (58) showed that plants can not only absorb EDTA, but also utilize the nitrogen of its molecule,

although this nitrogen was less effective than that of $NaNO_3$. The theory of "outer space in plants", of which Kramer's paper presents an excellent review (40), can explain satisfactorily how large molecules of EDTA can be absorbed by roots and leaves. Kramer suggests that unlike "accumulation", "absorption" does not mean an entrance against a concentration gradient; and therefore no energy exchange is involved in absorption. According to this conception, EDTA enters the "outer space" in the plant by a mere diffusion mechanism.

There are several theories about how iron is released from its complex within the plant after iron chelate has been absorbed. Hill-Cottingham (33) and Wallace et al (83) suggested two steps concerning this unsolved problem: a) sunlight decomposition of chelate; b) reduction of ferric iron to ferrous iron within the chelate and its subsequent replacement by other metals.

A number of investigations have indicated that EDTA and its metal salts, in high concentrations, are toxic to plants. The physiology of this toxic effect is not fully understood. Certain conclusions, however, have been drawn from results of enzyme studies. Gross (26) noted a stimulation of adenosine triphosphatase by the use of low concentration (.002M) of EDTA. He suggested that stimulation of enzyme action by EDTA in non-inhibitory concentrations results from the protection of the enzyme from traces of heavy metals which are toxic to the enzyme system. These

results indicate that there is a competition between EDTA and the enzymes in the plants for metals essential for enzyme activity. Weinstein et al (86) suggested that with increasing concentrations of Na_2EDTA, the activities of cytochrome oxidase and ascorbic acid oxidase in soybean decreased, whereas that of polyphenol oxidase increased. Bonner (7) reported that EDTA and other chelating agents could reactivate the succinic dehydrogenase-cytochrome-oxidase system following the inactivation of this system by various means.

Heath and Clark (29, 30) have shown that EDTA at very low concentrations acts as a growth substance and that 3-indoleacetic acid acts in the same "remarkable and symetrical" way as EDTA does in the plants. Bennet-Clark (4) indicated that EDTA concentration between $10^{-5}M$ and $10^{-3}M$ could act as a growth promoter in the straight growth test. Honda (36) reported interesting effects of ascorbic acid and metal-complexing agents on the respiration of barley roots.

From the preceding review it becomes evident that chelates have many unique and far-reaching possibilities as carriers of mineral nutrients for plants. Further evidence for this assertion will be found from the results to be presented here.

MATERIALS AND METHODS

Germination of seedlings and transplantation. Hybrid field corn (Zea mays L., Kingscrost, Ke) was chosen as the experimental plant. Seeds were germinated in wooden flats containing sand. The seedlings were transplanted to glazed crocks of 3.8 liters capacity (1 gallon) 6 days after planting, when the shoots were about 7 cm. tall. The most uniform plants were selected from a large number of seedlings. Roots were thoroughly washed in distilled water before transplantation.

The inside of each culture jar was painted with a waterproof varnish before use. Each jar was provided with a wooden cover which had in it two holes about 1 inch in diameter. Seedlings were placed in these holes and supported by cotton plugs (Fig. 2 and Plate 1). Special care was taken to leave a space of 1-2 inches between the lower side of the wooden cover and the surface of the solution. This was done in order to facilitate the growth of root hairs and proper aeration of roots (17).

Composition of the solution. The basic composition of the solutions used appears in Table I. Stock solutions of half-molar strength were prepared with distilled water. Proportional amounts of each stock solution were freshly combined at the start of the cultures and at each renewal of the solutions. Stock solutions of metal chelates were stored in the dark since they are known to deteriorate in

Table I

THE PRIMARY COMPOSITION OF THE SOLUTION PER LITER

Macro-salt	Concentration millimoles	Micro-salt	Concentration micromoles
$Ca(NO_3)_2$	0.40	H_2BO_3	8.3
$(NH_4)_2SO_4$	0.27	$MnCl_2$	4.0
NH_4NO_3	0.14	$ZnSO_4$	0.3
$MgSO_4$	0.33	$CuSO_4$	0.1
KH_2PO_4	0.26	$FeCl_3$*	62.0

*The solution of $FeCl_3$ was freshly prepared immediately before use, since any stock solution of this compound would deteriorate in contact with air.

FIG. 2 SECTIONAL VIEW OF A CULTURE JAR WITH PLANTS

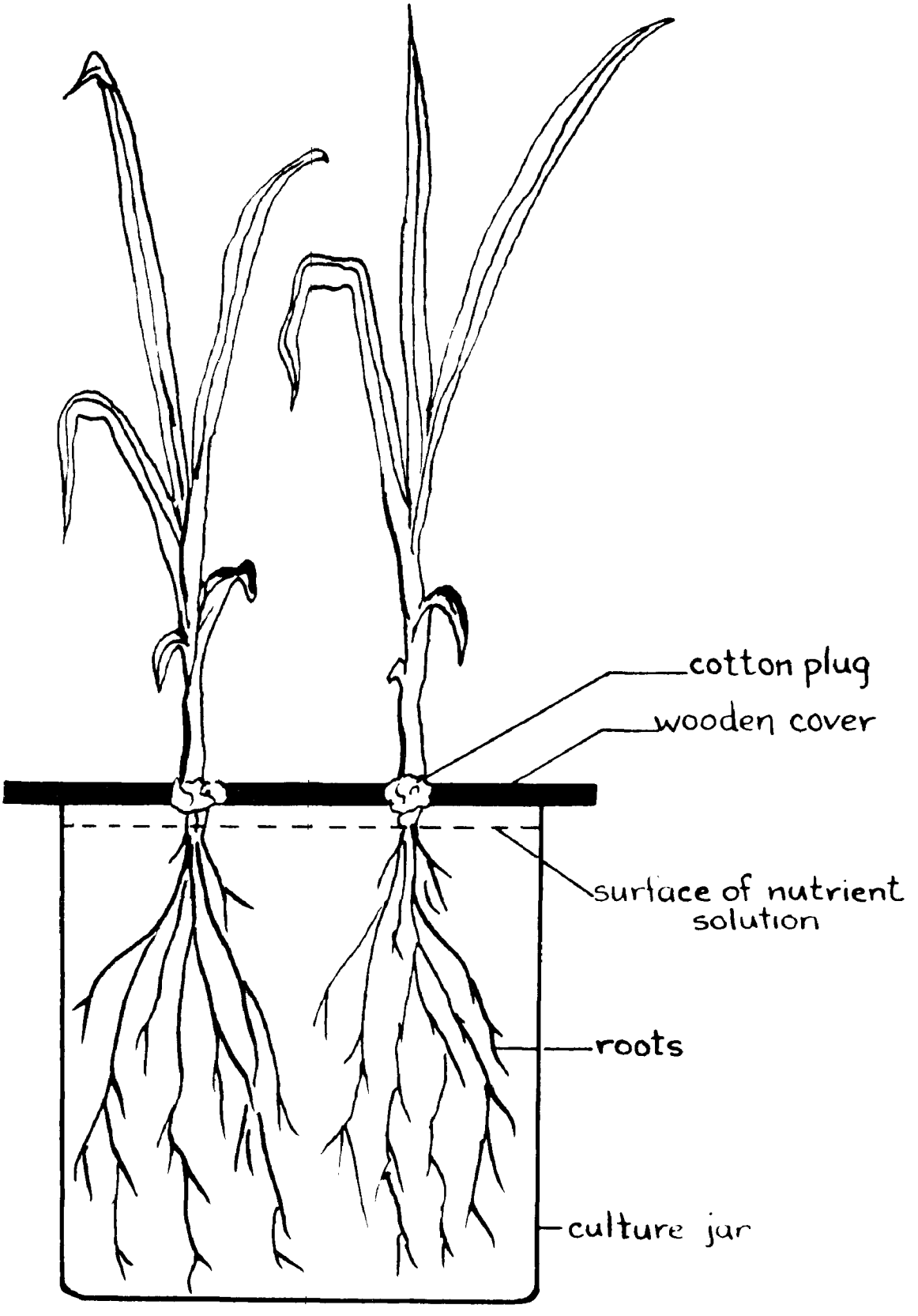


Plate 1

Set-up of nutrient culture in the greenhouse and the process of aeration of the solutions.

contact with light.

It will be seen later in this presentation that the composition of the solution is closely related to the purpose of each experiment. Certain modifications of the composition listed in Table I were found necessary. Any deviation from this general composition (Table I), however, will be mentioned and discussed in connection with the experiment concerned.

Recording of data and harvesting. Solutions were changed once every week and aerated once in the middle of the week. Since some variation in plants might result due to their different locations in relation to the sunlight and the heating unit of the greenhouse, culture jars were rearranged at intervals to minimize any such variation. There were five culture jars for each treatment, with two plants in each jar (unless otherwise stated). The general growth habit of the plants, including any symptoms of mineral deficiency, was noted from time to time. Hydrogen-ion-concentration of different solutions was determined colorimetrically with Eastman Universal Indicator and recorded on alternate days during the experiment. The greenhouse temperature was maintained at approximately 25°C. during the day and 20°C. at night.

Plants were harvested after 21 days from the date of transplantation. Each plant was divided into top and roots and was dried in a drying chamber (about 60°C.) for 2-3 days. Dry weights of tops and roots were taken as a measure of the effects of the different treatments. Statistical analysis was done on the basis of mean dry weight of

each pair of plants per jar. The ratio of top to root was determined in some experiments in order to indicate the nitrogen-supply (58); this is because higher nitrogen levels promote top growth more than root growth. Standard errors and statistical significance of data were calculated whenever it seemed necessary; it was done according to the method of T-test described by Paterson (57).

RESULTS AND DISCUSSION

Part I Sources of Iron and Phosphorus and their Interactions

Until the discovery of Fe-EDTA as a source of iron for plants growing in solution culture (49), the iron-supply was considered the most difficult problem in solution cultures. High concentration of phosphorus in the solution and a high pH of the solution are known to accentuate the difficulty of iron-supply. The following two experiments were designed to determine whether certain forms of organic phosphates[1] could substitute for potassium dihydrogen phosphate in solution cultures and whether they would interact with inorganic sources of iron in the same way as do the inorganic phosphates.

Experiment 1. In this experiment there were five treatments, each represented by 10 culture jars. The source of iron for each of the treatments was Fe-EDTA[2] (10 micromoles per liter). Treatment A, which had potassium dihydrogen phosphate as the source of phosphorus, was considered the control. Plants in treatment B received sodium tetraphosphate (0.26 millimoles per liter). Plants in treatment C, D, and E, on the other hand, received as the sources of

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[1]All the three organic phosphates used in this experiment were obtained from Monsanto Chemical Company.

[2]Obtained from Geigy Chemical Corporation.

phosphorus, ethyl ammonium phosphate, ethyl acid phosphate, and n-propyl acid phosphate respectively. The concentrations of each of these organic phosphates were 0.48 millimoles per liter of culture solution. The source of potassium in treatments B, C, D, and E was potassium chloride (0.21 millimoles per liter). Other constituents of the solutions in all the treatments were the same as listed in Table I. Plants were harvested after 21 days from the date of transplantation. Average dry weights of tops and roots in all the treatments were compared with those in treatment A (control). The range of pH of the culture solutions in all the treatments was between 4.0 and 5.0. The results are shown in Table 2 and Fig. 3.

Properties of the organic phosphates employed. Ethyl ammonium phosphate is a water-white to yellow-tinged liquid of ammoniacal odor (specific gravity 1.23 at 25°C.). Chemically it is a mixture of 35% monoethyl diammonium phosphate, 50% diethyl ammonium phosphate, and 15% monoethyl ammonium hydrogen phosphate. Thirty-eight percent of this compound is phosphorus calculated as P_2O_5.

Ethyl acid phosphate is a colorless liquid of fairly high viscosity (specific gravity 1.30 at 25°C.). Fifty-one percent of this compound is phosphorus calculated as P_2O_5.

N-propyl acid phosphate is a yellowish liquid with a specific gravity of 1.20 at 25°C. Forty-five percent of this compound is phosphorus calculated as P_2O_5.

Table II

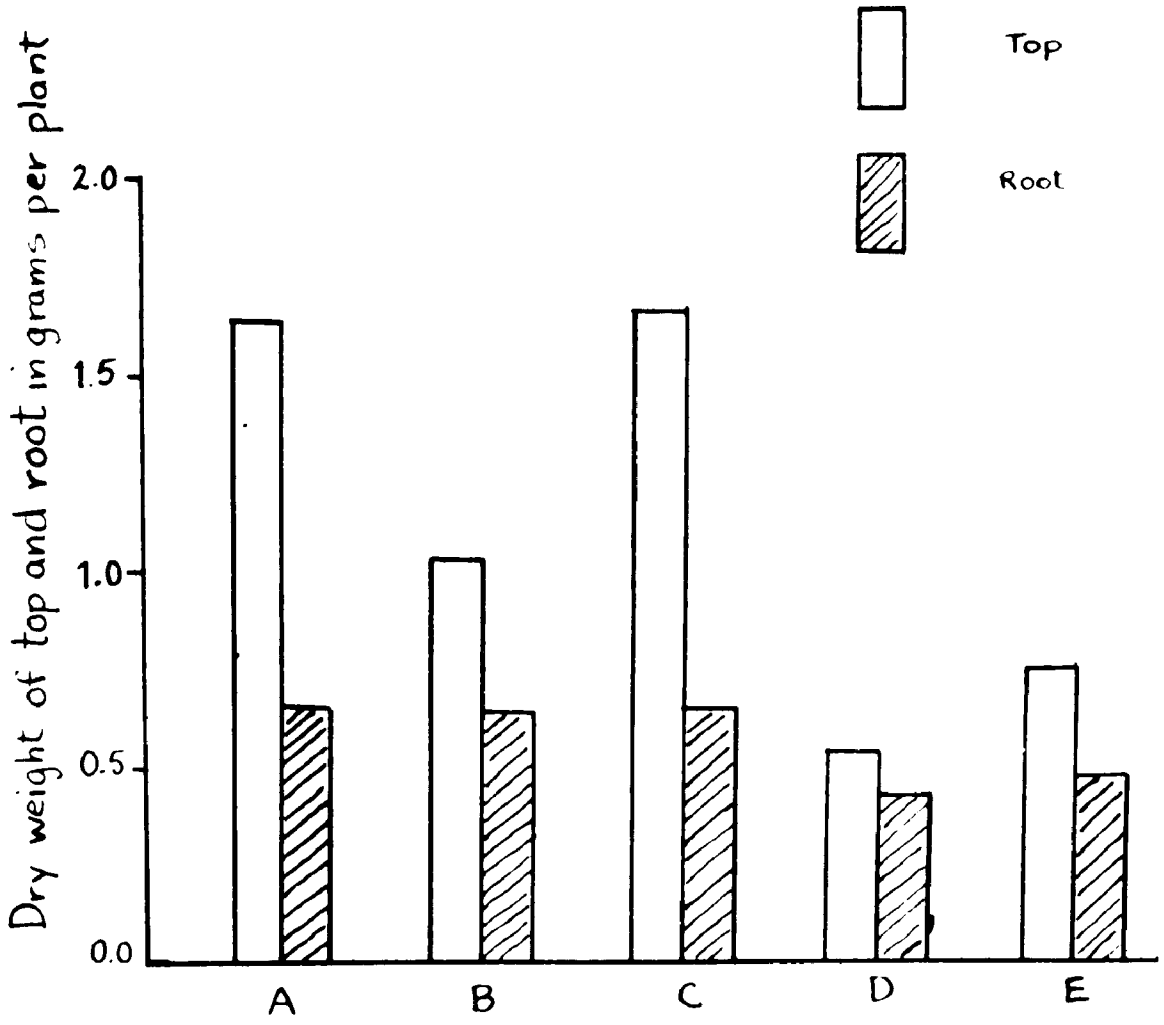
THE EFFECT OF ORGANIC PHOSPHATES ON THE GROWTH OF CORN IN SOLUTION CULTURE AS COMPARED TO THAT OF INORGANIC PHOSPHATES

Treatments	Concentration of P, millimoles per liter	Dry weight grams per plant		Standard error	
		Top	Root	Top	Root
A. Potassium dihydrogen phosphate (control)	0.06	1.63	0.63	0.086	0.047
B. Sodium tetraphosphate	0.07	1.00**	0.63	0.072	0.038
C. Ethyl ammonium phosphate	0.06	1.62	0.60	0.085	0.033
D. Ethyl acid phosphate	0.14	0.49**	0.41**	0.060	0.022
E. N-propyl acid phosphate	0.13	0.75**	0.48*	0.047	0.013

*Significantly less than the corresponding values of both treatments A and C at the 0.05 level.

**Significantly less than the corresponding values of both treatments A and C at the 0.01 level.

FIG. 3 EFFECT OF DIFFERENT FORMS OF PHOSPHORUS ON THE GROWTH OF CORN IN SOLUTION CULTURE



A. KH_2PO_4; B. Sodium tetraphosphate; C. Ethyl ammonium phosphate; D. Ethyl acid phosphate; E. n-propyl acid phosphate.

It is evident from the results shown in Table II and Fig. 3 that plants receiving ethyl ammonium phosphate (treatment C) grew as well as those of the control (treatment A). There were no visible symptoms of mineral deficiency in either of the two treatments. The average dry weights of shoots in treatments B, D, and E, however, were significantly less than those of treatments A and C. The growth of roots in treatments D and E also was distinctly inhibited as compared to that of treatments A and C.

Plants in treatment B showed a considerable amount of intervenal chlorosis at the time of harvest, although they grew equally good root systems as plants in treatments A and C. This is in partial agreement with Drumheller (21) who suggested that Na-tetraphosphate compared favorably with KH_2PO_4 as a source of phosphorus in nutrient solution and that it promoted better root growth . Plants in treatments D and E were stunted; and the leaf blades were yellow in color, while the bases of the blades and the sheaths were purple. These visual symptoms are known to be due to phosphorus deficiency (84). Such symptoms occurred in treatments D and E in spite of the fact that the plants here received about twice as much phosphorus as the plants in the other treatments (Table II). This indicates that the phosphorus from ethyl acid phosphate and n-propyl acid phosphate is not totally available to plants growing in solution cultures.

The effective use of ethyl ammonium phosphate in solution culture--as is evident from the normal growth of

plants in treatment C--raises two relevant questions: a) How does the phosphorus of this compound become available to plants?, and b) Can this compound be used in a solution culture of higher pH in which the source of iron is inorganic? The answer to the first question is beyond the scope of the present investigation. The following experiment was designed, however, to gain information pertaining to the second question which has an important bearing on the problems to be discussed later in this presentation.

Experiment 2. There were five treatments in this experiment. Each treatment consisted of five culture jars. Plants in treatment A--the control--received precisely the same solution as the control in the previous experiment. In treatment B ethyl ammonium phosphate and ferric chloride (Table I) were used as sources of phosphate and iron respectively. In treatment C the sources of both iron and phosphorus were inorganic in form (Table I). Iron of any form was omitted from treatment D. Treatment E, on the other hand, was totally free from any trace of phosphorus. The source of potassium in treatments B and E was potassium chloride (0.21 millimoles per liter). The other constituents of the solutions in all the treatments were the same as listed in Table I.

Plants were harvested after 21 days from the date of transplantation. The pH of the solutions in all the treatments was maintained between 6.0 and 7.0 by adding minute amounts of HCl or NaOH to the solutions. Results

are shown in Table III, Fig. 4, and Plates 2a, 2b, and 3.

The results indicate that there is a distinct relation between the sources of iron and phosphorus in the solution and the growth of plants. There was very slight difference in growth--statistically insignificant--between the plants in treatment A and those in treatment B. They were equally vigorous in appearance and developed no visual symptoms of iron or phosphorus deficiency (with treatments D and E as criteria of such deficiency). Plants in treatment C, on the other hand, were very stunted and chlorotic (Plates 2b and 3) in appearance, which is indicative of iron deficiency. They were, however, free from any symptoms of phosphorus deficiency--purple color of the leaf base and midrib.

The iron chlorosis in the plants of treatment C may be attributed to the fact that Fe^{+++} of $FeCl_3$ is progressively precipitated out by KH_2PO_4 at a high pH and becomes unavailable to the plants. This is in substantial agreement with previous workers (25, 38, 18). Various organic forms of iron, such as ferric tartrate, ferric citrate, and Fe-EDTA, have been employed in order to overcome this problem (61, 72, 37, 32, 23). The result of the present experiment (treatment B) provides substantial evidence to support the conclusion that the problem of iron-supply in nutrient culture can be overcome by using ethyl ammonium phosphate. This organic phosphate does not seem to interact with $FeCl_3$ in the same way as does KH_2PO_4.

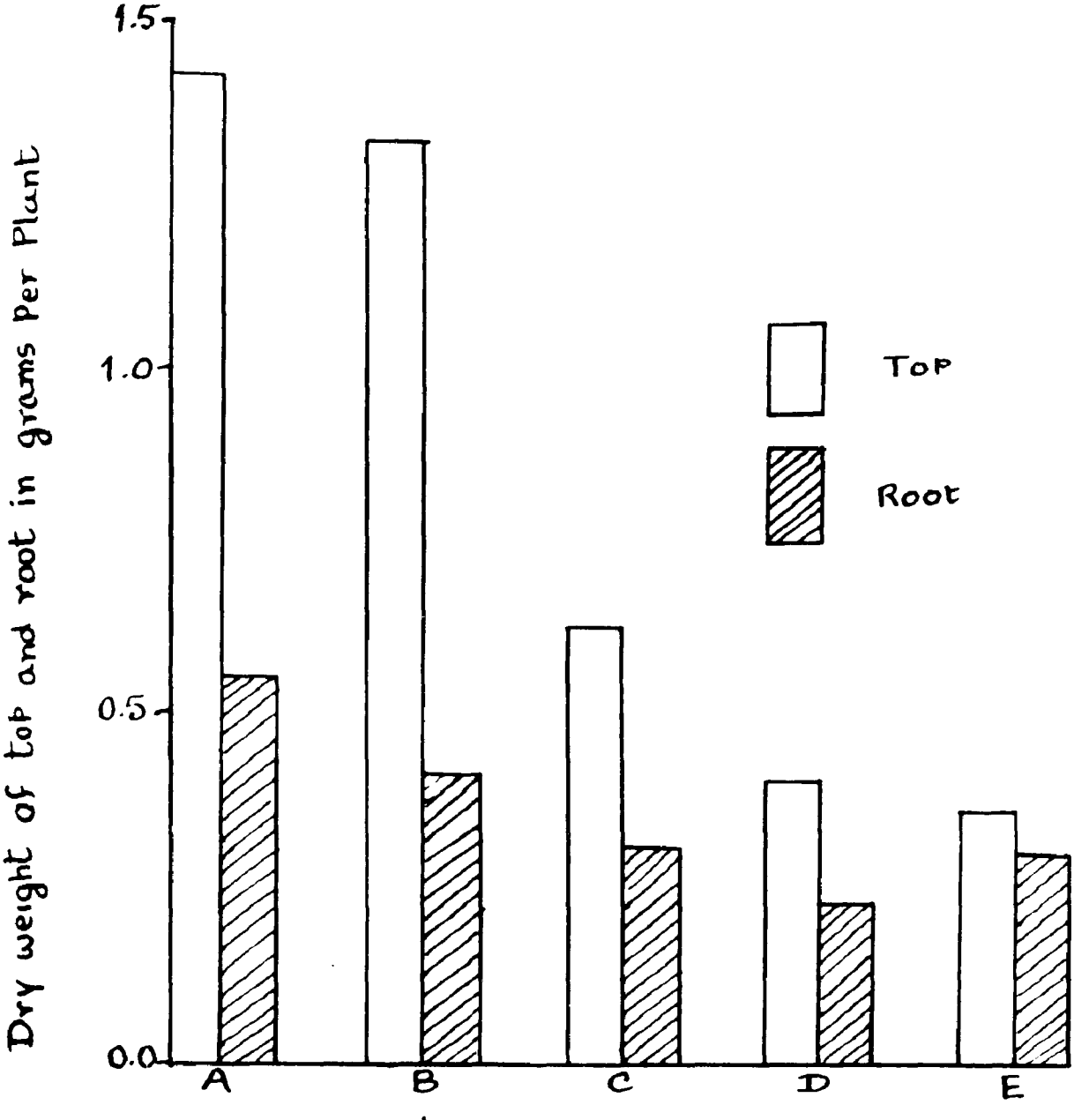
Table III

COMPARATIVE EFFECTS OF
ORGANIC AND INORGANIC SOURCES OF PHOSPHORUS AND IRON
UPON THE GROWTH OF CORN IN SOLUTION CULTURE

Treatments	Dry weight grams per plant		Standard error	
	Top	Root	Top	Root
A. Fe-EDTA and KH_2PO_4	1.42	0.54	.063	.039
B. $FeCl_3$ and Ethyl ammonium phosphate	1.31	0.40	.077	.015
C. $FeCl_3$ and KH_2PO_4	0.63**	0.30**	.055	.008
D. KH_2PO_4 and no iron	0.39**	0.23**	.037	.017
E. $FeCl_3$ and no phosphorus	0.36**	0.30**	.031	.005

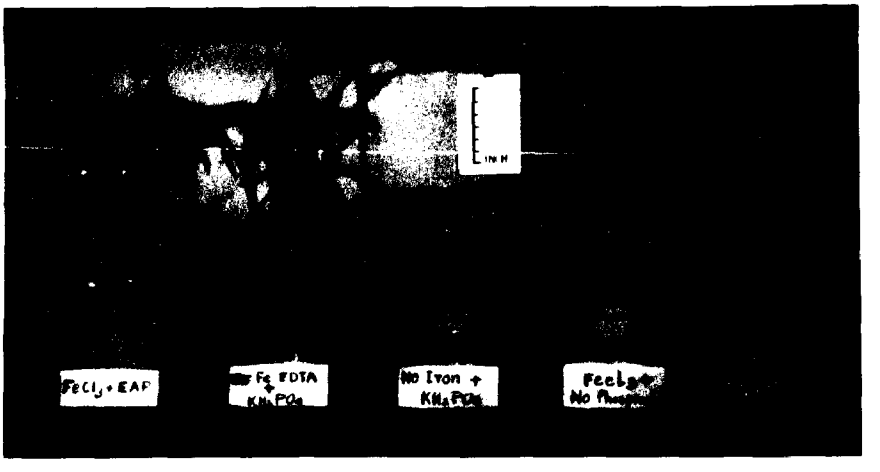
**Significantly less (at 0.01 level) than the corresponding values of both treatments A and B.

FIG. 4 EFFECT OF IRON-PHOSPHORUS INTERACTION ON THE GROWTH OF CORN



A. KH_2PO_4 and Fe-EDTA; B. Ethyl ammonium phosphate and $FeCl_3$; C. KH_2PO_4 and $FeCl_3$; D. No Iron; E. No Phosphorus.

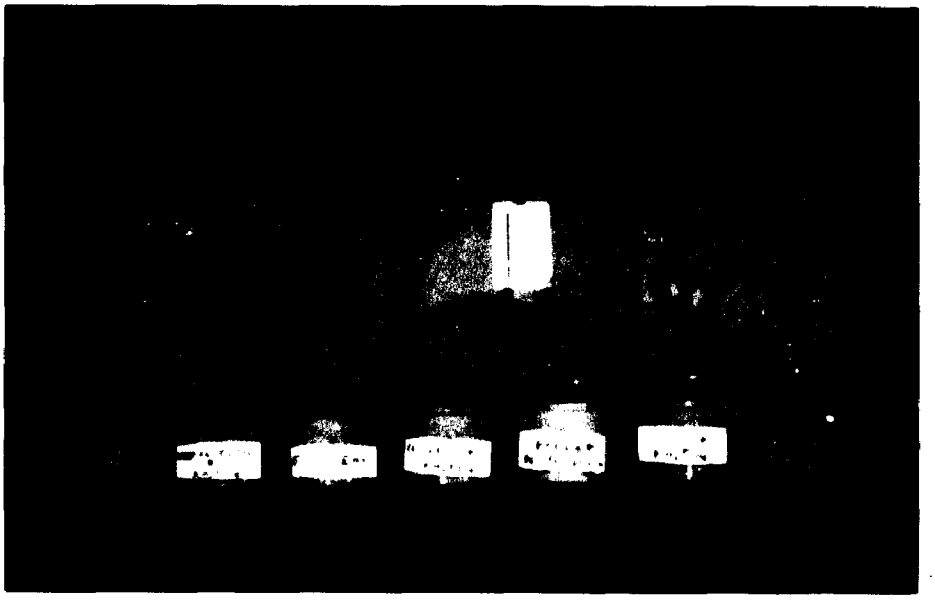
Plate 2a



Interaction of different sources
of phosphorus and iron
in solution culture.

EAP stands for Ethyl Ammonium Phosphate.

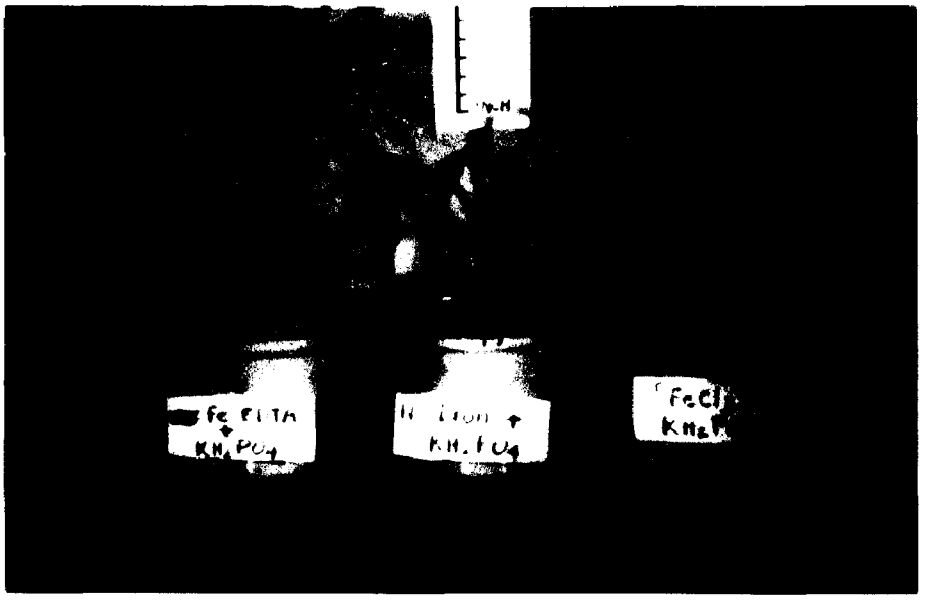
Plate 2b



Sources of iron and phosphorus
and their interactions
in solution culture.

Left to right: Treatments A, B, D, E, and C.

Plate 3



Iron-Phosphorus interaction
in solution culture.

There appears to be no published account of successful use of any organic phosphate in solution culture. Spencer and Willhite (70) found a number of organic phosphates equally as available to plants as inorganic phosphates when applied to soil. Edgerton (23) used sodium metaphosphate in combination with iron tartrate for apple plants growing in solution culture. Dunn and Roberts (22) successfully used magnesium propyl-phosphate as a source of magnesium for apple plants in solution culture. Since they used potassium dihydrogen phosphate as the major source of phosphorus, it will need further investigation to determine whether the phosphorus from magnesium propyl-phosphate was utilized by the plants. It seems probable that the magnesium compound furnished a relatively small part of the total phosphorus used by the plants in this instance.

Part II Ca-acetate as a Source of Calcium and its Advantages

Hydrogen-ion concentration of a nutrient solution has an important bearing on the availability of nutrient elements in general and metal chelates in particular. The solutions used in the previous experiments were not buffered, and frequent adjustments of the pH were necessary--particularly toward the end of the growth period. The following experiment was designed in order to determine whether calcium acetate--an effective buffer--could substitute for calcium nitrate (Table I) as a source of calcium in the culture solutions.

Experiment 3. There were two treatments, each consisting of five culture jars. Plants in treatment A received calcium acetate (0.26 millimoles per liter) instead of calcium nitrate. They also received sodium nitrate (0.40 millimoles per liter) which compensated for the loss of nitrate nitrogen caused by the withdrawal of calcium nitrate. The sources of potassium and phosphorus in both treatments were potassium chloride (0.21 millimoles per liter) and ethyl ammonium phosphate (0.48 millimoles per liter) respectively. Other constituents of the solution in both treatments were the same as listed in Table I.

The hydrogen-ion concentrations of the solutions were recorded every day during the experiment. No attempt was made to adjust the pH of the solutions artificially.

Results are shown in Table IV and Fig. 5. Figure 5 indicates that there was considerably less fluctuation in the pH of the solution in treatment A (Ca-acetate series) as compared to that of treatment B (Ca-nitrate series). The pH of both solutions at the start of the experiment, and also at each renewal of the solutions, was 6.0. In treatment A this was the lowest pH-value recorded during the whole growth period, and the range of pH in this treatment remained between 6.0 and 7.0. In treatment B, however, the solution was more acidic, and the range of pH remained between 4.0 and 5.0 during the experiment. From this result it becomes obvious that the pH of a culture solution can be kept consistently high by using Ca-acetate instead of Ca-nitrate.

Plants in both treatments were healthy in appearance and without any symptoms of mineral deficiencies. The data on dry weight yields (Table IV) indicate that plants in the Ca-acetate series not only grew as well as those in the Ca-nitrate series, but also grew more luxuriant root systems than the latter. A comparison between the ratios of top to root in both treatments will make this more evident. This shows that Ca-acetate can be used as a source of calcium in a solution culture.

Dunn and Roberts (22) successfully used magnesium acetate as a source of magnesium for corn in solution culture. This compound showed a buffering effect similar to that of calcium acetate. Michael (53) found that uptake of calcium

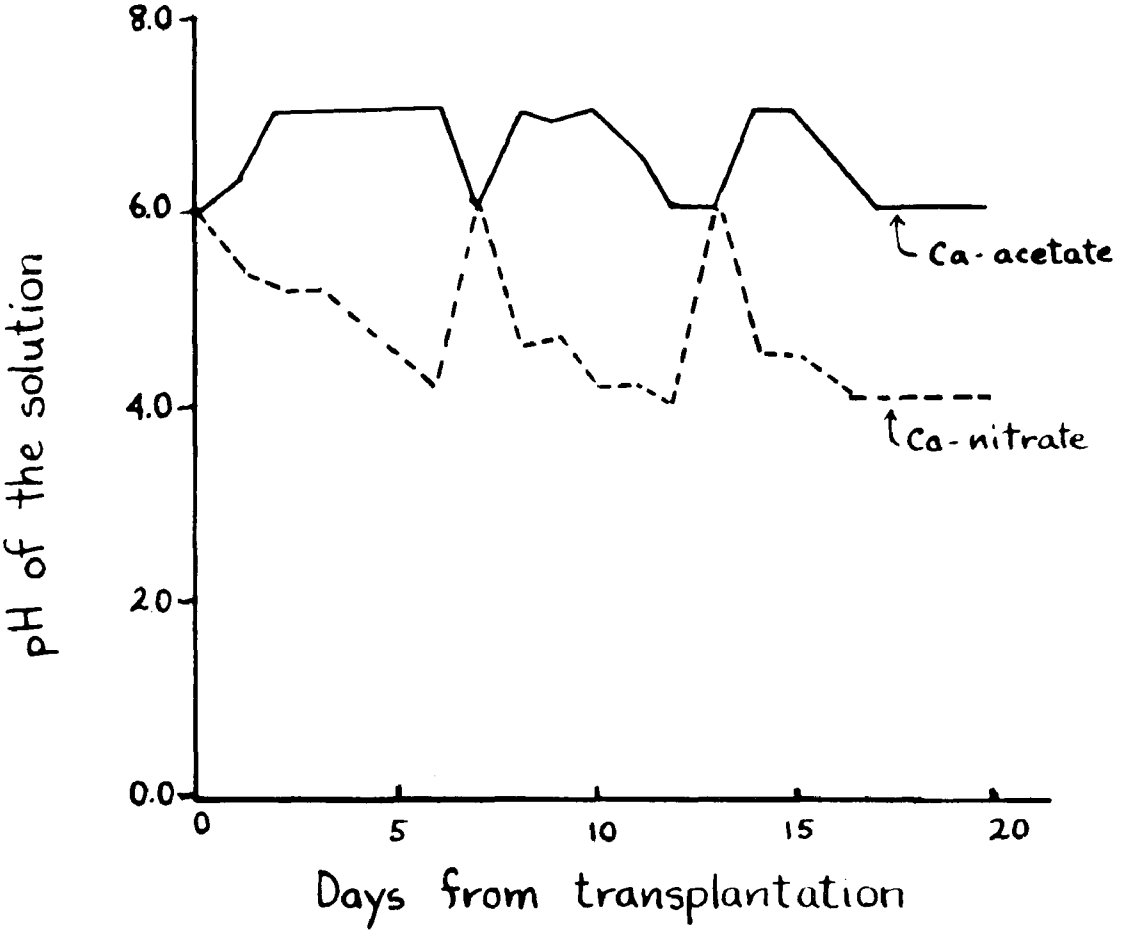
Table IV

EFFECT OF CALCIUM ACETATE ON THE GROWTH OF CORN IN SOLUTION CULTURE

Treatments	Dry weight grams per plant Top	Root	Standard error Top	Root	Dry weight Top / Root
A. Calcium acetate	1.49	0.69**	.054	.033	2.16
B. Calcium nitrate	1.51	0.39	.093	.035	3.87

**Significantly higher (at 0.01 level) than the corresponding value of the other treatment.

FIG.5 THE EFFECT OF CALCIUM ACETATE ON THE pH OF CULTURE SOLUTION



from culture solutions by young corn and rye plants was considerably lower at pH 4.0 than at pH 6.0 or 7.5. Smith (65) studied the growth of citrus seedlings with nitrate and ammonium forms of nitrogen in solution cultures and observed a significant reduction in growth--irrespective of the form of nitrogen--at pH 4.0 as compared to pH 6.0. Since the concentrations of nitrate nitrogen (.091 millimoles per liter) and ammonium nitrogen (.092 millimoles per liter) in the solutions of both treatments in this experiment were exactly the same, the effective use of Ca-acetate as a source of calcium may be attributed to the higher pH (6.0-7.0) of the solution in treatment A.

It is known that metal chelates with relatively lower stability constants, such as calcium and magnesium chelates, tend to dissociate in a solution of lower pH. In view of the fact that such metal chelates will be employed in the forthcoming experiments, the author found it justified to modify the composition of the solution listed in Table I in the light of the results of the foregoing experiments. The modified composition of the solution to be used hereafter appears in Table V.

Table V

MODIFIED COMPOSITION OF THE SOLUTION PER LITER

Macro-salt	Concentration millimoles	Micro-salt	Concentration micromoles
Ca-acetate	0.26	H_2BO_3	8.3
$NaNO_3$	0.40	$MnCl_2$	4.0
$(NH_4)_2SO_4$	0.27	$ZnSO_4$	0.3
NH_4NO_3	0.14	$CuSO_4$	0.1
$MgSO_4$	0.33	$FeCl_3$*	62.0
KCl	0.21		
Ethyl ammonium phosphate	0.48		

*The solution of $FeCl_3$ was freshly prepared immediately before use, since any stock solution of this compound would deteriorate in contact with air.

Part III Experiments on EDTA and its Metal Complexes

Experiment 4. This experiment was concerned with the effects of the disodium salt of EDTA[1] on the growth of corn in solution culture (45). The composition of the solutions in all the treatments was identical with that listed in Table V except for the varying amounts of Na_2EDTA. There were five culture jars per treatment. The total growth period was the same as in the previous experiments. The range of pH of the solutions in all treatments was between 6.0 and 7.0. The results are shown in Table VI, Figs. 6 and 7, and Plates[2] 4a, 4b, and 5.

It appears from the results that there is a definite correlation between the amounts of Na_2EDTA in solution and plant growth, which was progressively better with decreasing concentrations. Amounts of 80 micromoles or higher were sooner or later lethal to the plants. At 200 micromoles concentration, the plants died immediately after transplantation. There was no increase in dry weight at this concentration. For this reason the weights of these plants were taken as a base and were subtracted from the mean dry weights in all other treatments to show the mean dry weight increase (columns 5 and 6, Table VI). Plants receiving 160 micromoles

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[1]Obtained from Geigy Chemical Corporation.

[2]Concentrations in ppm, as appear in the plates, may be converted into micromoles per liter by multiplying them with 0.8.

Table VI

EFFECT OF DIFFERENT CONCENTRATIONS OF EDTA
ON THE GROWTH OF CORN IN SOLUTION CULTURE

Na_2EDTA micromoles per liter	Mean dry wt. grams per plant		Standard error		Mean dry weight increment after transplantation		Mean dry weight increment as % of control	
	Top	Root	Top	Root	Top	Root	Top	Root
0.0	1.53	0.61	0.109	0.045	1.40	0.49	100	100
2.5	1.90	1.02**	0.096	0.062	1.77	0.89	126.7	182.4
5.0	1.75	0.91**	0.088	0.094	1.62	0.79	115.7	160.5
10.0	1.67	0.91*	0.109	0.045	1.54	0.78	110.2	160.3
20.0	0.67	0.43			0.54	0.31	38.9	63.0
40.0	0.40	0.28			0.27	0.15	19.4	31.3
80.0	0.29	0.23			0.16	0.11	11.7	21.7
120.0	0.18	0.16			0.05	0.04	3.8	7.4
160.0	0.14	0.13			0.01	0.01	0.9	1.6
200.0	0.13	0.12			0.0	0.0	0.0	0.0

*Difference compared with control significant at 0.05 level.

**Difference compared with control significant at 0.01 level.

FIG. 6 THE EFFECT OF VARYING CONCENTRATIONS OF Na_2EDTA ON THE GROWTH OF CORN IN SOLUTION CULTURE

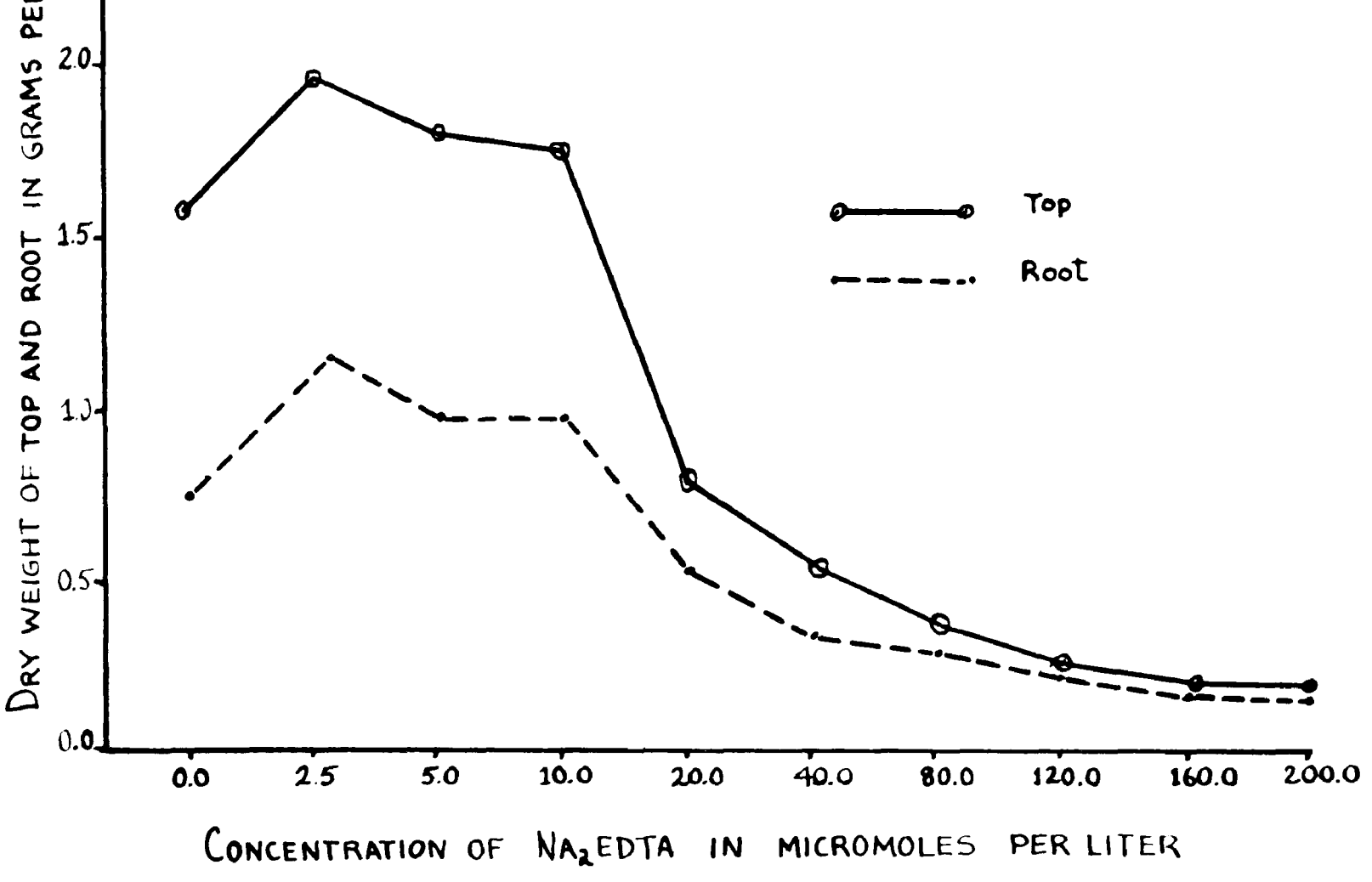


FIG. 7 EFFECT OF VARYING CONCENTRATIONS OF Na_2EDTA ON THE GROWTH OF CORN

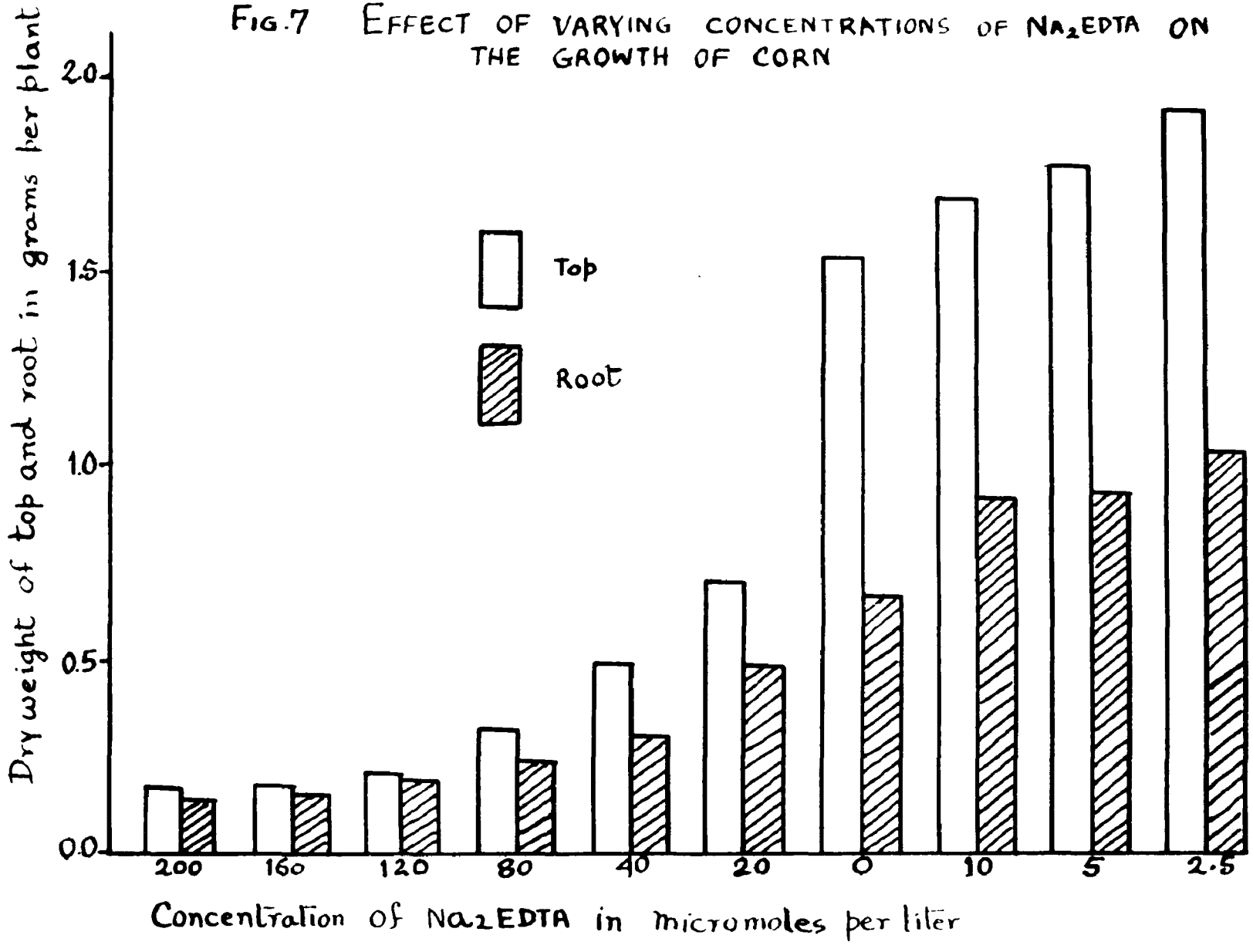


Plate 4a

Effect of varying concentrations of EDTA
on the growth of corn
in solution culture.

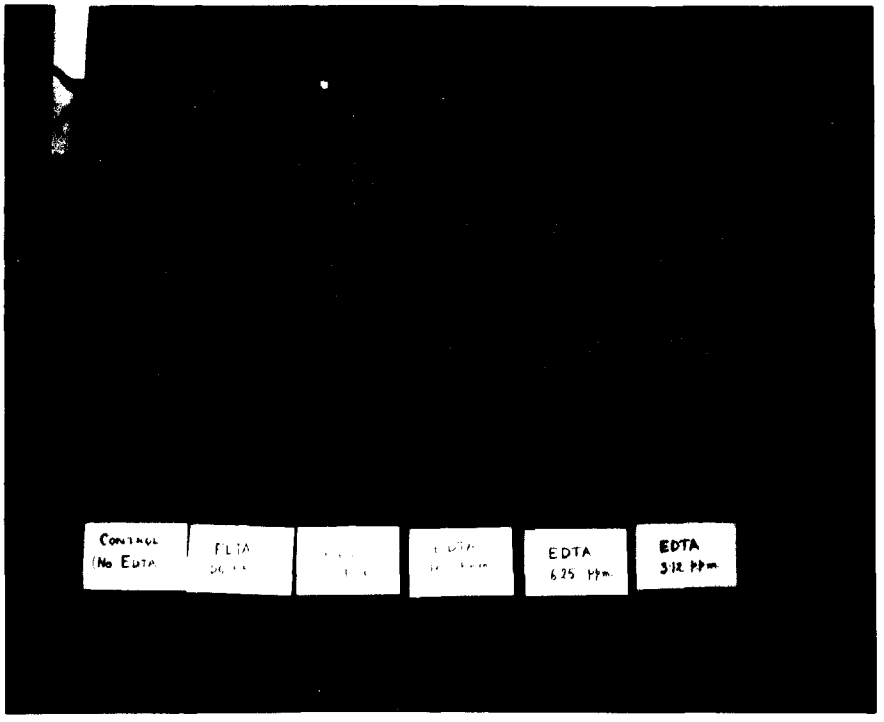
Left to right: concentrations of EDTA are
0.0, 40.0, 20.0, 10.0, 5.0, 2.5 micromoles per liter.

Plate 4b

Effect of varying concentrations of EDTA
on the growth of corn
in solution culture.

Left to right: concentrations of EDTA are
0.0, 40.0, 20.0, 10.0, 5.0, 2.5 micromoles per liter.

Plate 5



Effect of varying concentrations of EDTA
on the root growth of corn
in solution culture.

Left to right: concentrations of EDTA are
0.0, 40.0, 20.0, 10.0, 5.0, 2.5 micromoles per liter.

of EDTA survived for about a week, whereas those receiving concentrations of 120 micromoles and 80 micromoles died in about 8 and 12 days respectively from the date of transplantation. At 40 and 20 micromole levels plants were very chlorotic and stunted (Plates 4a and 4b). Concentrations of 10 micromoles, 5 micromoles, and 2.5 micromoles not only were non-toxic, but appeared to be growth-promoting as compared to the control which contained no EDTA. It seems from this that the upper non-toxic level of EDTA concentration approximated 10 micromoles per liter. It may also be noted that plants receiving non-toxic concentrations of EDTA grew more luxuriant root systems than those without this compound (Table VI and Plate 5).

The chelating agent (Na_2EDTA), when mixed with a solution of various inorganic salts, forms complexes with metals in an order depending on the stability of the corresponding complexes. It forms more stable compounds with iron and copper than with any other metals, and chelated iron has been successfully used as a source of iron for plants (38, 72, 85, 88). In connection with the present experiment it may be noted, however, that at all levels of Na_2EDTA below 20 micromoles, the molar ratio of EDTA to Fe was less than 1:1. This means that at these levels all the Fe^{+++} ions were not chelated. Therefore, Fe from $FeCl_3$, rather than from Fe-EDTA, was probably the primary source of iron for the plants. Furthermore, the supply of iron was adequately insured even without the addition of EDTA

to the solution since ethyl ammonium phosphate did not form precipitates of iron phosphate with ferric chloride (results of experiments 1 and 2). Therefore, the beneficial effect of low concentrations of EDTA on plants growing in solution culture under the conditions of this experiment must be attributed to a growth-promoting property of this compound.

Heath and Clark (29, 30) have suggested that EDTA at very low concentrations acts as a growth substance, and that 3-indole acetic acid affects growth in the same way as a chelating agent. Recently this has been contradicted by Burström and Tullin (14). Ford et al (24) reported that soil applications of iron chelates increased the growth of feeder roots in citrus plants. Weinstein et al (86) found that 10 ppm of EDTA was optimum for soybeans grown in solution cultures. Results by Jacobson (38) on corn were in substantial agreement with this.

Gross (26) reported an increase in activity of adenosine triphosphatase at lower concentrations of EDTA, but a significant inhibition in its activity at higher concentrations. He suggested that the stimulation of enzyme action by EDTA in non-toxic concentrations results from the "protection of the enzyme" from traces of heavy metals such as copper and manganese which are inhibitory to certain enzyme systems. Whether this would be a factor in the beneficial effect of low concentrations of EDTA on plants grown in solution culture is a question needing further investigation. The following two experiments were designed

to gain information on copper and manganese toxicity as modified by EDTA of a non-toxic concentration.

Experiment 5. There were two sets of six treatments, each treatment consisting of five culture jars. The treatments of one set received six different concentrations of $CuSO_4$. The other set was identical with the first one except for the 5 micromoles of EDTA in each of its treatments. Other constituents in all the treatments were the same as listed in Table V. The growth period was the same as in the previous experiments. The hydrogen-ion concentration in all treatments remained between 6.5 and 7.0 throughout. Results are shown in Table[1] VII, Figs. 8, 9, 10, and 11, and Plates[1] 6a, 6b, 7, 8, and 9.

It is very evident from the results that EDTA can modify copper toxicity in corn to a considerable extent. Plants receiving 0.1 and 0.2 micromoles of $CuSO_4$ did not show any visible abnormality in growth regardless of the presence or absence of EDTA in the solution (Plate 6a). With increasing concentrations of copper, the growth of plants receiving no EDTA progressively deteriorated (Plate 6b) and was accompanied by severe chlorosis and browning of roots which are known to be symptoms of Cu-toxicity (89). Plants receiving EDTA, however, showed no indication of copper toxicity although there was a trend of depression

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[1]The designations x1 Cu, x2 Cu, x4 Cu, x8 Cu, and x10 Cu, as appear in the table and plates indicate respectively, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 micromoles of $CuSO_4$ per liter.

in growth--less drastic in nature--at higher concentrations of copper in the solutions (Table VII). Furthermore, the dry weight yields of most of the treatments receiving EDTA were significantly higher than those of the corresponding counterparts without EDTA. Plates 7, 8, and 9 will show that even at high concentrations of copper, EDTA promotes the growth of root systems considerably (45).

A number of workers have suggested that high concentrations of copper or manganese in the growth medium may cause iron-chlorosis in plants growing in that medium. Steinberg (71) reported that in culture solutions a relative freedom from toxic quantities of trace-element impurities was "conducive to good iron nutrition". Brown and Holmes (12) used catalase activity as an index of active iron in the plant and noted that this activity was comparatively higher in copper-deficient plants than in those with sufficient copper. Reuther and Smith (62) suggested that excessive accumulation of copper in the soil is the major factor for iron-chlorosis in Florida citrus groves. DeKock (19) reported that in the presence of ferric versenate in the nutrient solution, heavy metal toxicity is greatly reduced. Westgate (89) reported that soil acidity aggravates the iron-chlorosis induced by high copper concentration. He also noted that chelated iron, either as a foliar spray or as a soil amendment may correct iron-chlorosis even in the presence of a high concentration of copper. Smith and Specht (67) observed that excess quantities of Cu, Zn, or Mn in the nutrient

Table VII

MODIFYING EFFECT OF EDTA[1] ON THE COPPER TOXICITY IN CORN GROWN IN SOLUTION CULTURE

Treatments	Mean dry weight grams per plant		Standard error	
	Top	Root	Top	Root
x1 Cu	2.09	1.16	.133	.081
x2 Cu	2.11	0.98	.088	.073
x4 Cu	1.35	0.80	.157	.057
x6 Cu	0.66	0.42	.093	.052
x8 Cu	0.48	0.38	.025	.031
x10 Cu	0.43	0.36	.039	.018
x1 Cu and EDTA	2.40	1.24	.229	.090
x2 Cu and EDTA	2.73	1.35*	.232	.073
x4 Cu and EDTA	2.97**	1.44**	.118	.075
x6 Cu and EDTA	2.26**	1.09**	.143	.087
x8 Cu and EDTA	2.24**	1.08**	.151	.064
x10 Cu and EDTA	1.64**	0.84**	.140	.059

[1]The concentration of EDTA was 5 micromoles per liter or 6.25 ppm throughout.

* Significantly higher (at 0.05 level) than the value in the corresponding treatment receiving no EDTA.

**Significantly higher (at 0.01 level) than the value in the corresponding treatment receiving no EDTA.

FIG. 8 THE EFFECT OF COPPER TOXICITY ON THE SHOOT-GROWTH OF CORN AS MODIFIED BY EDTA

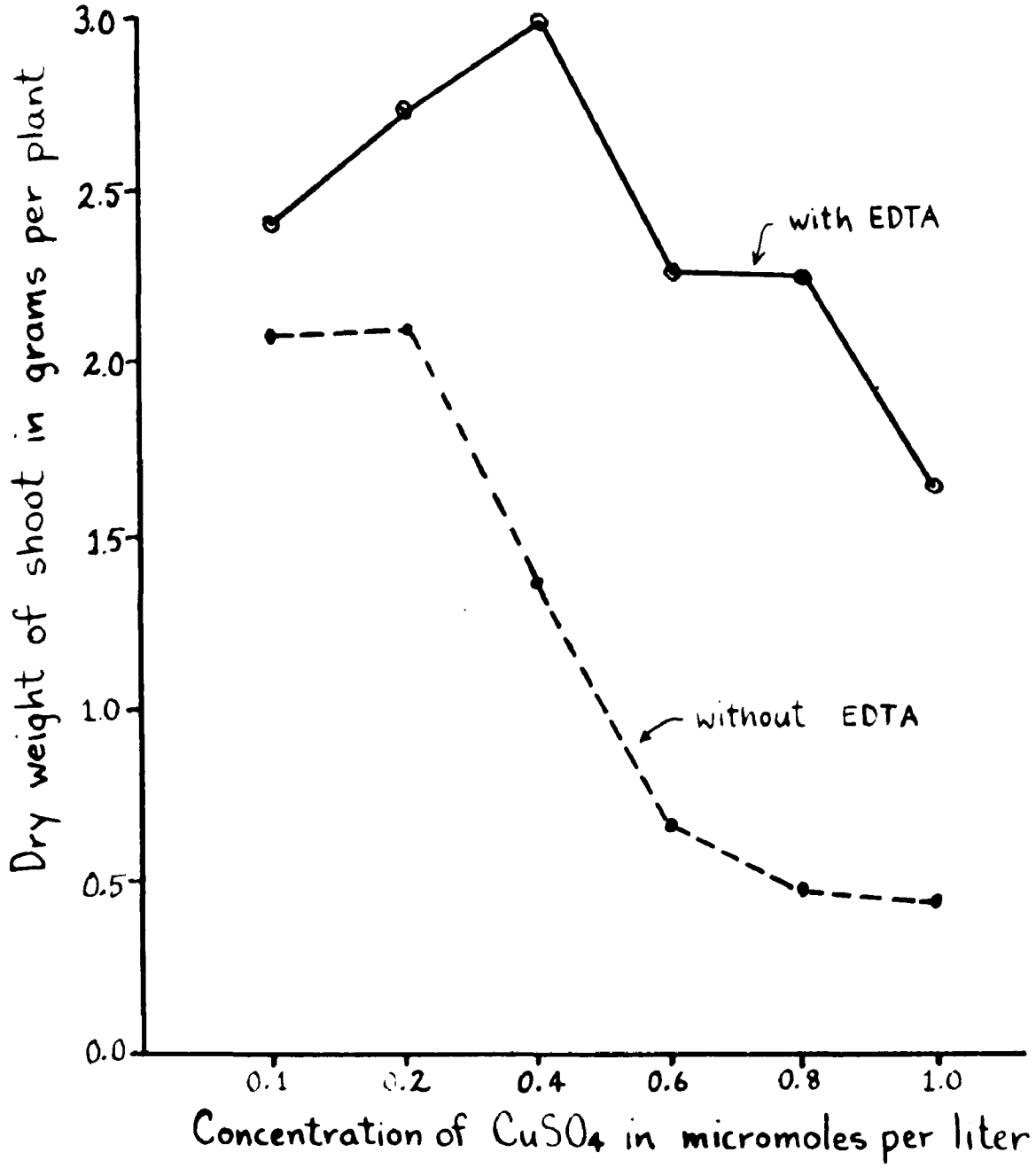


FIG. 9 THE EFFECT OF COPPER TOXICITY ON THE ROOT GROWTH OF CORN AS MODIFIED BY EDTA

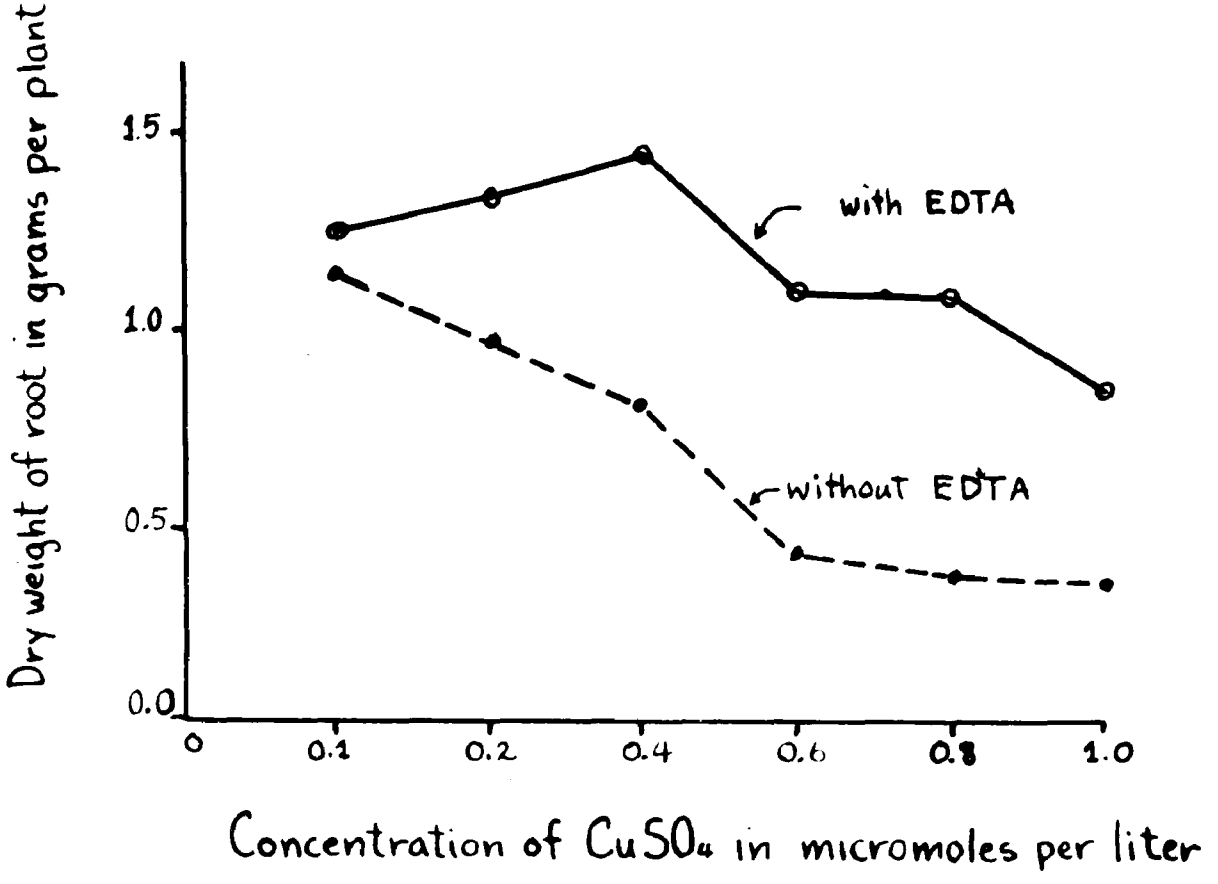


FIG. 10 COPPER TOXICITY IN CORN SHOOT AS MODIFIED BY EDTA

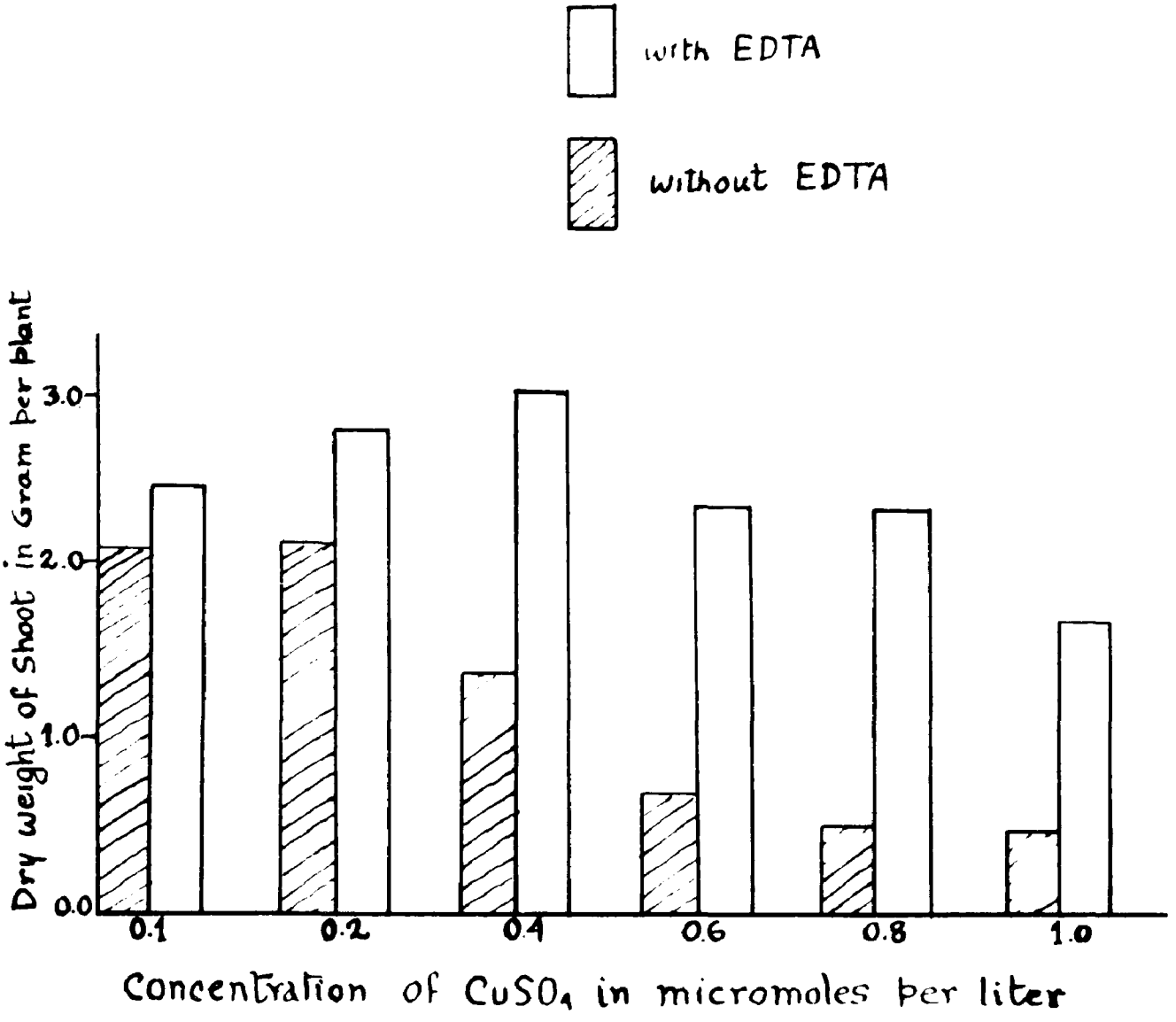


FIG. 11 COPPER TOXICITY IN CORN ROOTS AS MODIFIED BY EDTA

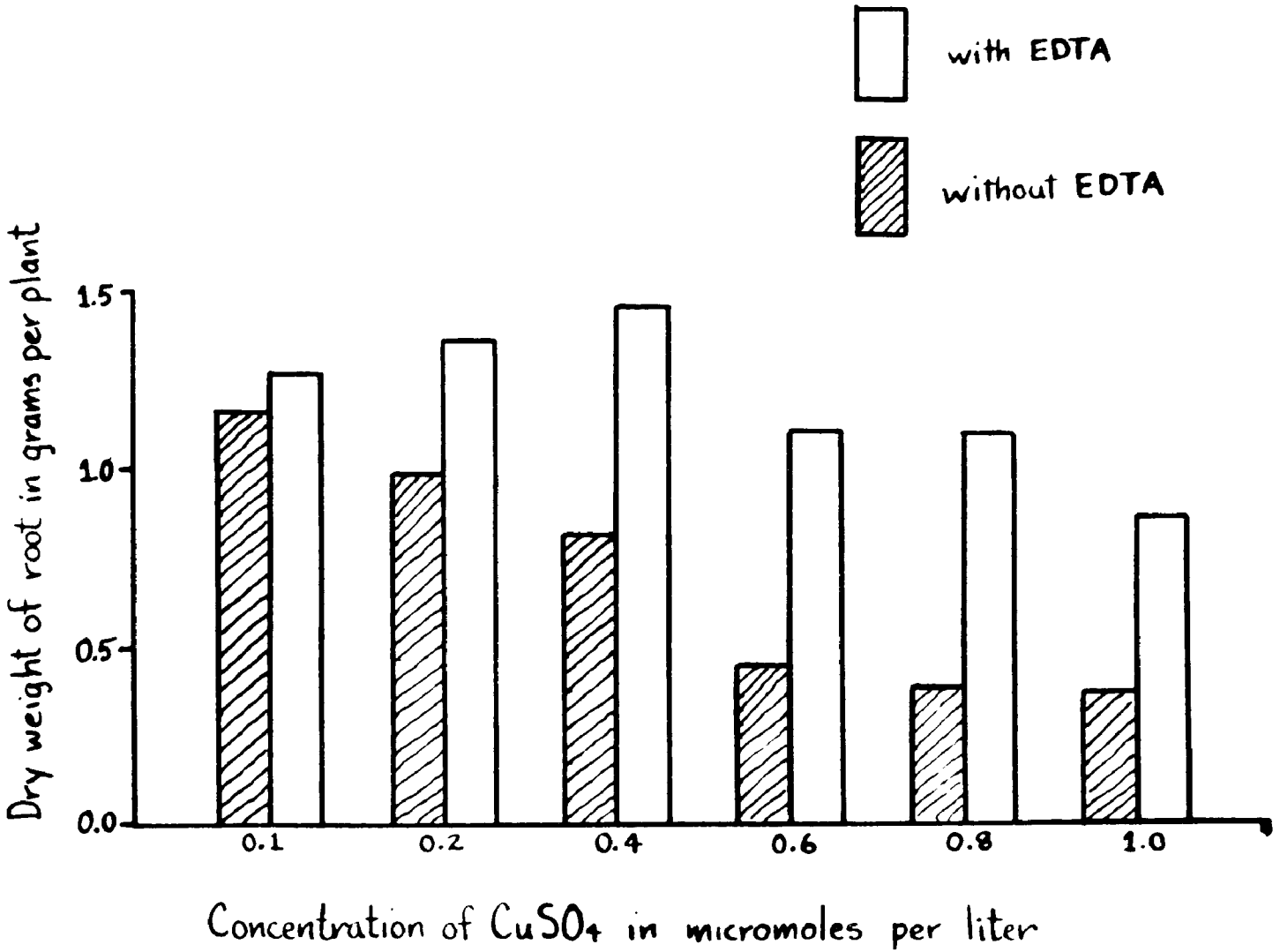
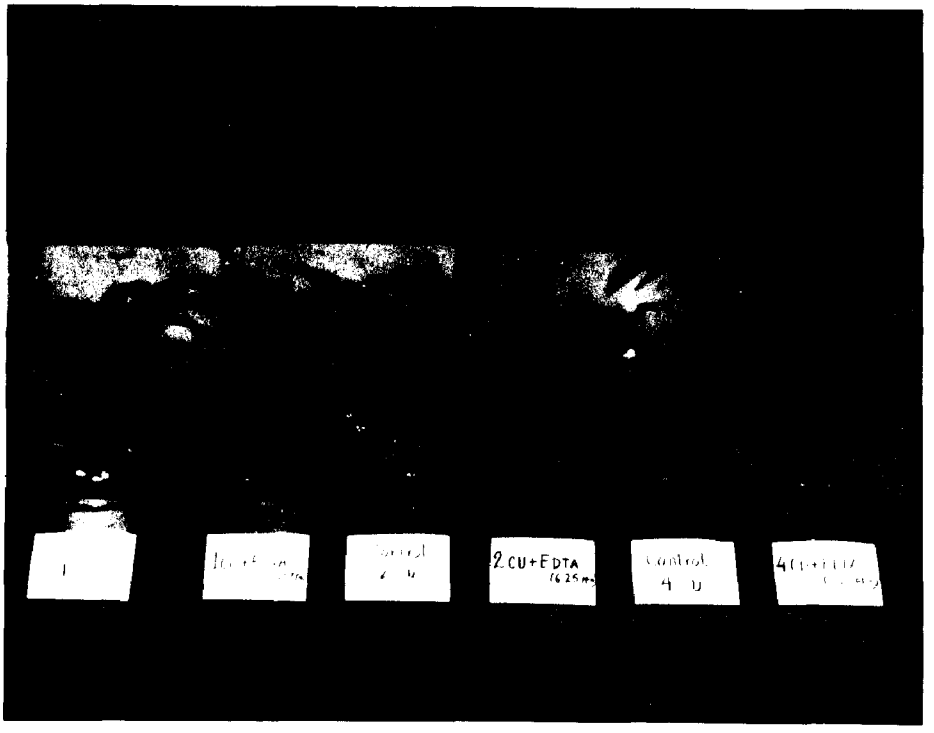
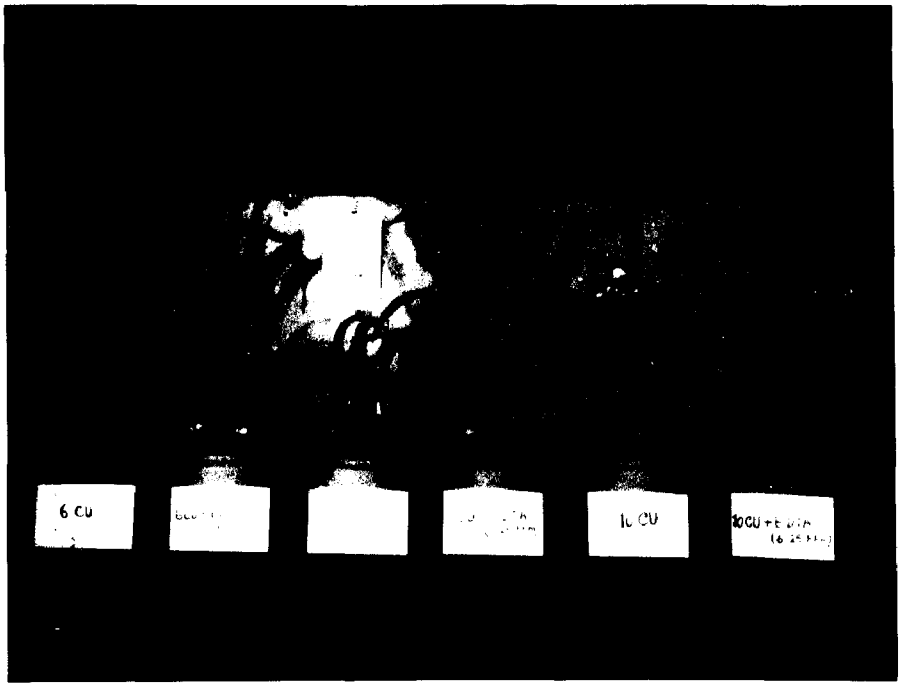


Plate 6a



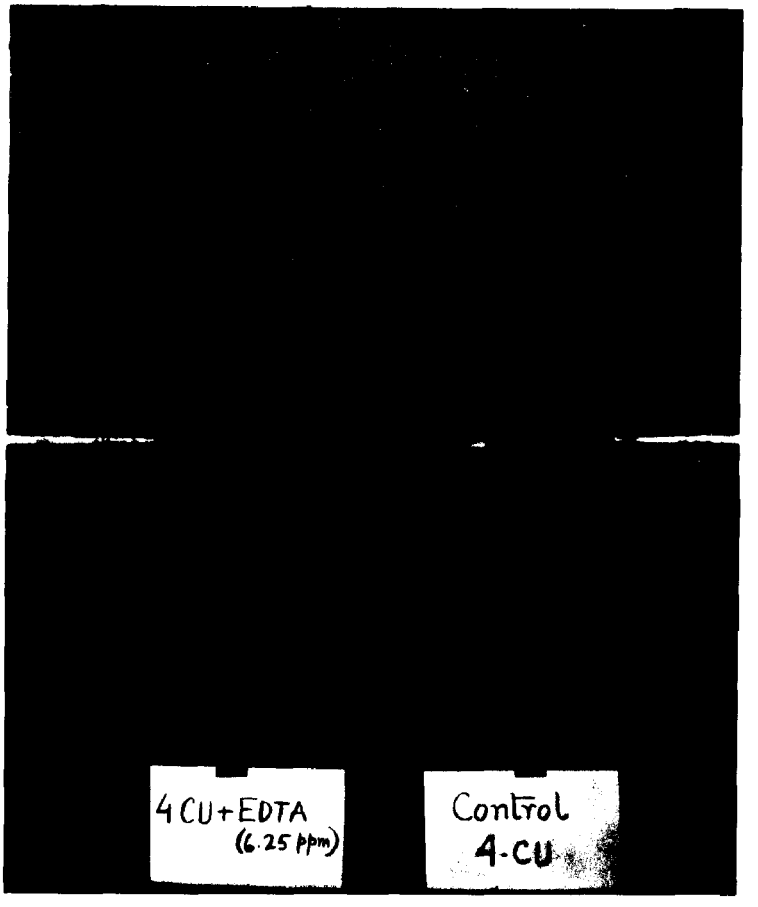
Modifying effect of EDTA
upon the copper toxicity in corn
grown in solution culture.

Plate 6b



Modifying effect of EDTA
upon the copper toxicity in corn
grown in solution culture.

Plate 7

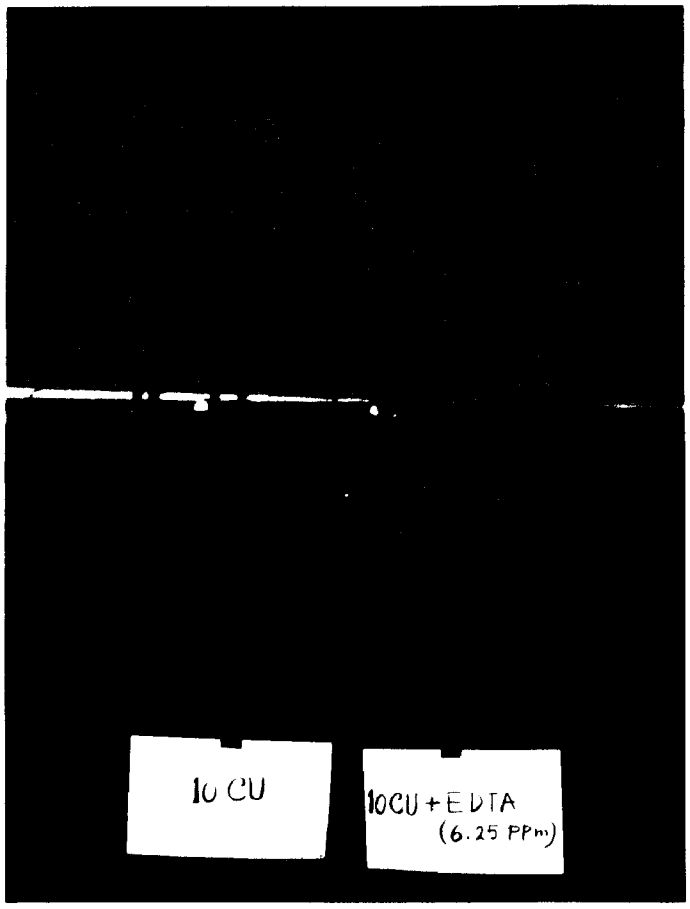


Effect of 0.4 micromoles of $CuSO_4$
on the root growth of corn
as modified by EDTA.

Plate 8

Effect of 0.8 micromoles of $CuSO_4$
on the root growth of corn
as modified by EDTA.

Plate 9



Effect of 1.0 micromoles of $CuSO_4$ on the root growth of corn as modified by EDTA.

solution caused stunting and chlorosis of citrus seedlings. They also found that the addition of chelated iron prevented chlorosis but did not improve the root systems. The results of the present experiment are in partial contradiction to the observation of Smith and Specht (67) since here EDTA proved effective in improving the root systems even at high copper concentrations (Plates 7, 8, and 9).

The biochemical basis for the modifying effect of EDTA on copper toxicity in solution culture is open to speculation. Rasmussen (59) concluded that "microelemental chelates may be added to nutrient solutions in somewhat higher concentrations than the corresponding salt without the microelements becoming toxic to the plants". Metal ions vary in the stability of the complexes they form with EDTA; and in a scale descending from strong to weak chelates, the order is ferric ion, copper, zinc, ferrous ion, manganese, calcium, and magnesium. Therefore, under the conditions of the present experiment, it is logical to assume that the metal ions which were chelated by EDTA were ferric ions and not cupric ions. Furthermore, there was not enough EDTA in the solution to chelate all the ferric ions as well as the cupric ions since the molar ratio of EDTA to Fe^{+++} ions in the solutions was less than 1:1. Hence, in order to explain the modifying effect of EDTA upon the Cu-toxicity, it becomes necessary to assume that all the ferric ions in the solution were reduced to ferrous ions by certain chemical interactions (33) before cupric ions were chelated by EDTA.

The ratio of EDTA to Cu^{++}, even in the treatment receiving the highest concentration of copper, was considerably higher than 1:1. In the event that the reduction of ferric ions occurs in the solution, all the cupric ions in all the treatments receiving EDTA should be chelated. Results presented here (Figures 8, 9, 10, and 11) indicate, however, that there was a gradual depression in the growth of the plants at concentrations higher than 0.4 micromole of $CuSO_4$ in the solutions in spite of the addition of EDTA. This shows that complete chelation of cupric ions in the solution is not necessarily a corrective for the copper toxicity, and that high concentrations of copper can interfere with the effectiveness of EDTA as a growth-stimulant, as shown in experiment 4.

Experiment 6. In this experiment there were two sets of three treatments each, with five culture jars in each treatment. The three treatments in the first set received different concentrations of manganese in their solutions. Treatments in the second set were identical with those of the first except for 10 micromoles of EDTA in each of them. Other constituents of the solutions in all the treatments were the same as listed in Table V. The hydrogen-ion concentration of all the solutions remained between 6.5 and 7.0 throughout the growth period of twenty-one days. Results are shown in Table VIII.

It appears from the results that the addition of EDTA to the solution did not prevent the chlorosis caused

Table VIII

EFFECT OF EDTA[1] ON THE Mn-TOSICITY OF CORN IN SOLUTION CULTURE

Treatments	Dry weight grams per plant Top	Root	Visual characteristic
x6 Mn	0.93	0.36	Normal and healthy
x8 Mn	0.86	0.32	Slight chlorosis
x10 Mn	0.79	0.32	Considerable chlorosis
x6 Mn and EDTA	0.75	0.31	Slight chlorosis
x8 Mn and EDTA	0.74	0.31	Considerable chlorosis
x10 Mn and EDTA	0.50	0.26	Stunted and chlorotic

[1]The concentration of EDTA was 10 micromoles per liter of culture solution.

The designations x6 Mn, x8 Mn, and x10 Mn as appear in the table above indicate 6.0, 8.0, and 10.0 micromoles of $MnCl_2$ per liter.

by high concentrations of manganese. Plants receiving 6 micromoles of $MnCl_2$ alone (without any EDTA) showed normal growth; but plants in all other treatments--regardless of the presence or absence of EDTA in the solution--were progressively stunted and chlorotic with the increasing concentrations of Mn^{++} in the solution. This deterioration in growth was more noticeable in the plants receiving EDTA.

The following points may be considered in explanation of the results. Previous workers have emphasized the importance of iron-manganese relation in plant metabolism. Somers and Shive (68) pointed out that in the plant tissue a high concentration of soluble iron is always associated with a low concentration of soluble manganese and vice versa. Perkins and Purvis (58) reported that the soil application of Mn-EDTA increased the amount of water soluble and exchangeable iron in the soil. In connection with the present experiment the author believes that iron-chlorosis resulted from the failure of EDTA to chelate excess Mn ions in the solution. This failure of chelation is due to the fact that EDTA does not form any complex with Mn as long as there are free Fe^{+++}, Cu^{++}, Zn^{++}, and Fe^{++} ions in the solution. The concentration of 10 micromoles is considered to be the upper non-toxic level for EDTA (result of experiment 4) in culture solution; and any increase of this concentration in order to chelate all the metal ions mentioned above as well as the Mn^{++} ions would be obviously detrimental to the plants. Therefore, under the conditions of this experiment, it may be concluded that the addition of non-

toxic quantities of EDTA can not prevent the Mn-toxicity of corn in solution cultures.

Part IV Experiments on "Versenol" and its Metal Complexes

Malcolm (46) considered "Versenol"[1] iron chelate as the most effective source of iron to correct the chlorosis in plants growing in alkaline soil. Rasmussen (59) suggested that chlorosis in plants grown in a nutrient solution of high pH could be corrected by the addition of "Versenol" iron chelate. The following three experiments were designed to gain further information concerning the use of "Versenol" and its magnesium and calcium chelates in nutrient culture.

Experiment 7. This experiment was concerned with the effect of different concentrations of "Versenol" on the growth of corn in solution culture. The composition of the solution of all treatments was the same as listed in Table V except for the varying amounts of "Versenol". Hydrogen-ion concentrations of the solutions in all treatments remained between 6.5 and 7.0 throughout the growth period of 21 days. Results are shown in Table IX, Fig. 12, and Plates 10 and 11.

Both Table IX and Fig. 12 show that there was very little difference in dry weight yields (statistically insignificant) between the plants in the control (without "Versenol") and those receiving 10 and 20 micromoles of "Versenol". There was a progressive deterioration in the growth of plants

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[1]"Versenol", obtained from Dow Chemical Company, is a tri-sodium salt of N-hydroxy ethyl ethylene diamine triacetic acid.

Table IX

EFFECT OF DIFFERENT CONCENTRATIONS OF "VERSENOL" ON THE GROWTH OF CORN IN SOLUTION CULTURE

Concentration of "Versenol" micromoles per liter	Dry weight grams per plant		Standard error	
	Top	Root	Top	Root
0	0.90	0.29	.062	.017
10	0.98	0.29	.055	.013
20	0.97	0.26	.058	.008
40	0.71	0.25	.085	.022
80	0.50	0.22	.049	.008
160	0.32	0.20	.030	.011

FIG. 12 EFFECT OF "VERSENOL" ON THE GROWTH OF CORN IN SOLUTION CULTURE

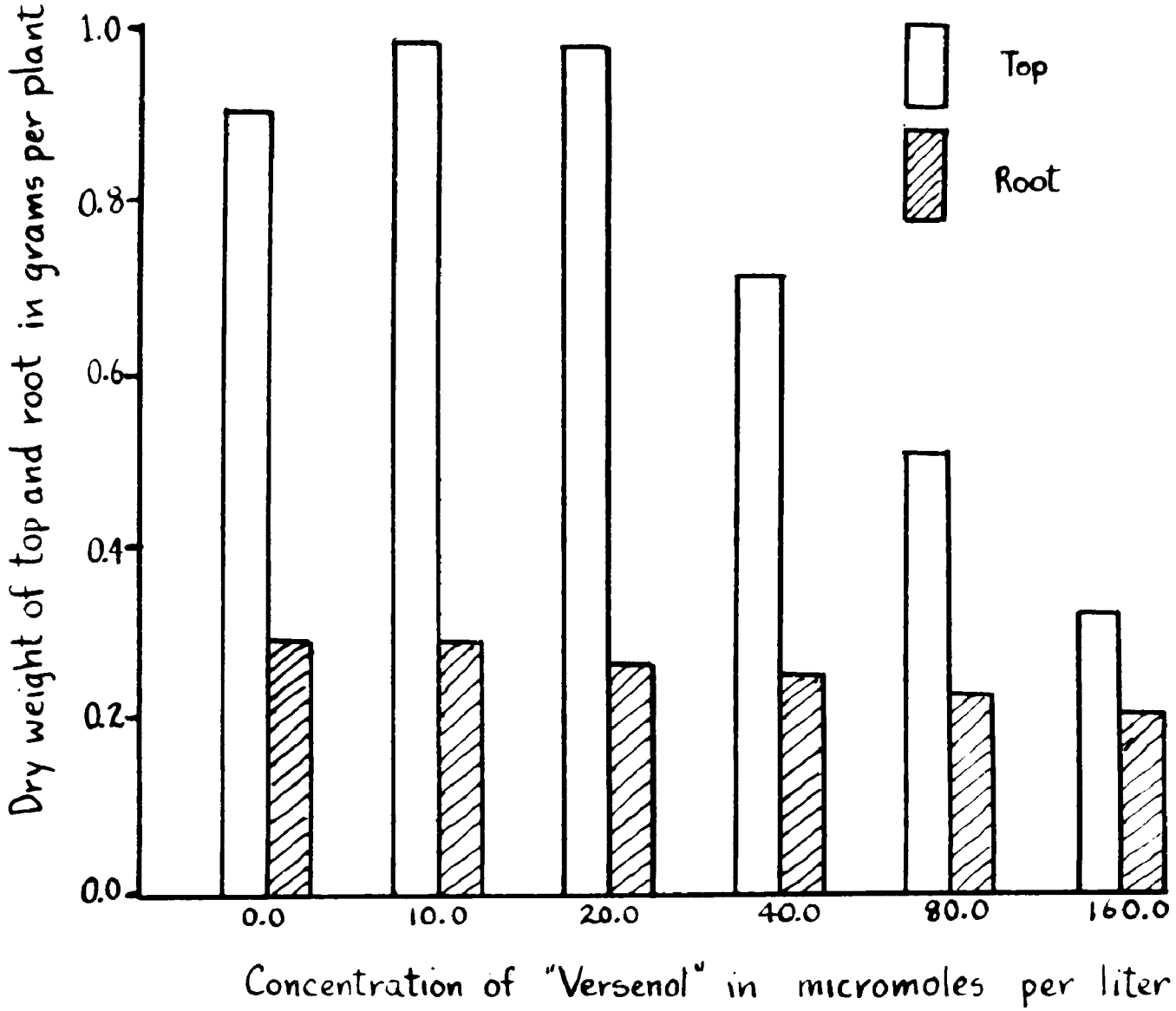

Plate 10

Effect of varying concentrations of "Versenol" on the growth of corn in solution culture.

Left to right: concentrations of "Versenol" are 0, 10, 20, 40, 80, 160 micromoles per liter.

Plate 11

Effect of "Versenol"
on the root growth of corn
in solution culture.

Left to right: concentrations of "Versenol"
are 0, 10, and 80 micromoles per liter.

with any further increase in concentrations of "Versenol", although this deterioration was less marked in root than in shoot (Fig. 12 and Plate 11). In appearance, however, the plants receiving 10 and 20 micromoles of "Versenol" looked somewhat more vigorous than those in the control (Plate 10).

It appears from the above considerations that the upper non-toxic level of "Versenol" concentration in nutrient culture approximates 20 micromoles per liter as against 10 micromoles for EDTA (experiment 4). EDTA in low concentration proved to be distinctly growth-promoting. The present experiment served only to determine the upper non-toxic limit for "Versenol" concentration in nutrient solution. It will be of interest, however, to determine the effect of "Versenol" concentrations lower than 10 micromoles on the growth of plants in solution culture.

Experiment 8. This experiment was designed to determine the possibility of chelated magnesium ("Versenol" magnesium chelate) as a source of magnesium in solution culture. Dunn and Roberts (22) found no beneficial effect of magnesium Versene[1] on corn and apple seedlings grown in solution cultures. Previous work in the Horticultural Department of the University of New Hampshire by R. Eggert (unpublished data) showed that Mg-EDTA in high concentrations was very toxic to plants both as foliar spray and

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[1]Magnesium Versene, Sequestrene Na_2Mg of Geigy Chemical Corporation.

soil applications.

In this experiment there were three treatments, each consisting of 10 culture jars. The source of iron in all treatments was "Versenol" iron chelate. Treatment A was considered to be the control since the plants here received magnesium sulfate (Table V) as the source of magnesium. In treatment B the only source of magnesium was "Versenol" magnesium chelate (6 micromoles per liter) which was prepared by combining pure "Versenol" and magnesium sulfate in equi-molecular proportions at pH 6.5, Treatment C, on the other hand, was kept free from any magnesium as far as possible. Other constituents of the solution in all treatments were the same as listed in Table V. The hydrogen-ion concentration of the solutions in all treatments was between 6.5 and 7.0 throughout the growth period of 21 days. Results are shown in Table X and Plate 12.

Plants in both treatments A and B looked very vigorous as compared to those in treatment C which were distinctly stunted and had all the symptoms of magnesium deficiency (chlorosis, necrotic leaf-spots, and poor root growth). The dry weight yields of tops and roots in treatments A and B were found to be significantly higher than those of treatment C. The difference between the dry weight yields of plants in treatment A and those in treatment B, however, was insignificant.

The following points may be considered in explanation of the results. The total concentration of "Versenol"

Table X

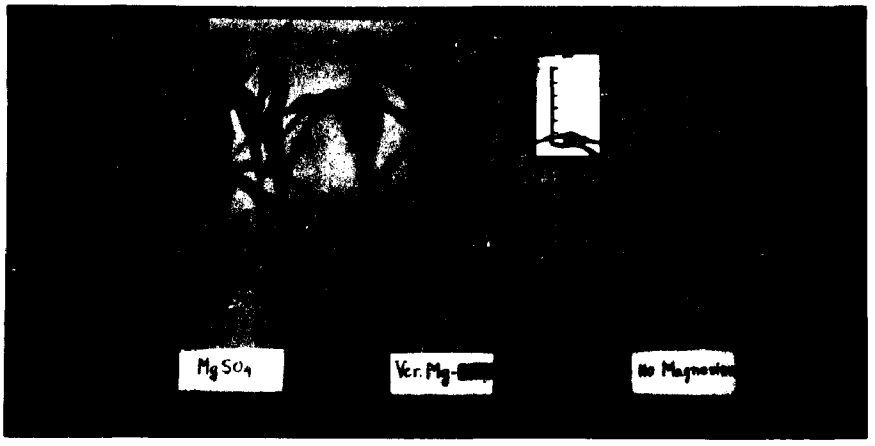
ROLE OF "VERSENOL" MAGNESIUM CHELATE
AS A SOURCE OF MAGNESIUM FOR CORN IN SOLUTION CULTURE

Metal Chelates, Micromoles per liter	Mean dry weight grams per plant		Standard error	
	Top	Root	Top	Root
A. "Versenol" iron chelate (9 micromoles)	0.94**	0.33**	0.015	0.006
B. "Versenol" magnesium chelate (6 micromoles) "Versenol" iron chelate (4.5 micromoles)	1.05*	0.38**	0.152	0.016
C. "Versenol" iron chelate (9 micromoles) No magnesium	0.55	0.25	0.034	0.008

*Significant at 0.05 level over the corresponding value in treatment C.

**Significant at 0.01 level over the corresponding value in treatment C.

Plate 12



Role of "Versenol" magnesium chelate
as a source of magnesium
in solution culture.

Ver-Mg is "Versenol" magnesium chelate
(6 micromoles)

metal chelate in any of the treatments did not exceed 10.5 micromoles which was found in previous tests to be the optimum concentration for "Versenol" under these conditions. The "Versenol" magnesium chelate in treatment B should dissociate in the solution to a very negligible extent throughout the experiment. This is because both iron and magnesium were in chelate form and there would be no possibility for the iron to replace magnesium from its complex. The percentage of magnesium as metal present in the "Versenol" magnesium chelate, however, was considerably lower than that in magnesium sulfate (treatment A). In spite of this low concentration of magnesium in treatment B, plants here did not show any signs of magnesium deficiency (with treatment C as index). Instead they were just as vigorous as those in treatment A. This indicates that magnesium of "Versenol" magnesium chelate was as readily utilized by plants as that of magnesium sulfate (45).

There appears to be no published account of the successful use of chelated magnesium as a plant nutrient in solution culture. It will be of considerable interest to employ techniques of chemical analysis in further evaluation of "Versenol" magnesium chelate as a source of magnesium for plants.

Experiment 9. This experiment was concerned with the possible use of "Versenol" calcium chelate as a source of calcium in solution culture. There were three treatments, each consisting of 10 culture jars. The source of iron for

the plants in all treatments was "Versenol" iron chelate. Treatment A was considered to be the control since the plants here received calcium acetate as the source of calcium (Table V). Plants in treatment B received "Versenol" calcium chelate (6 micromoles per liter) which was freshly prepared by combining calcium chloride and pure "Versenol" in equi-molecular proportions. Treatment C, on the other hand, was kept free from any form of calcium. The growth period was the same as in the previous experiments. The results are shown in Table XI and Plate 13.

Results indicate that "Versenol" calcium chelate is a poor substitute for calcium acetate as a source of calcium for corn in solution culture. During the first two weeks of growth, there was no visible difference between the plants in treatments A and B. At the end of the second week, however, plants in treatment B began to develop all the symptoms of calcium deficiency which were already manifest in the plants of treatment C. These symptoms included dying of the youngest leaf, involuted leaf margins, and brittleness of the growing points (84).

An examination of Plate 13 will indicate that, in spite of having involuted leaf margins typical of Ca-deficiency, the plants receiving "Versenol" calcium chelate were considerably more vigorous than those in treatment C. This suggests that the calcium metabolism in the plants of treatment B was not impaired from the beginning of the experiment as it was in the plants of treatment C. Therefore,

"Versenol" calcium chelate might have furnished a part of the total calcium requirement of the plants. It may be noted that the percentage of calcium as metal present in "Versenol" calcium chelate is considereably lower than that in calcium acetate (control); thus it may seem desirable to increase the concentration of "Versenol" calcium chelate in the nutrient solution in order to meet the total calcium requirement of the plants. The author, however, found in preliminary tests that any increase in concentration of "Versenol" calcium chelate beyond the 6 micromole level proved somewhat detrimental to the plants.

It may be noted that the pH of the solutions in treatments B and C was much lower than that of the solution in treatment A. Michael (53) found that the uptake of calcium by corn in solution culture was considerably less at pH 4.0 than at pH 6.0 or 7.5. Smith (65) reported that a reduced concentration of Ca^{++} ions in the foliage was associated with an increased pH of the solution. Steinberg (71), however, found that complete availability of calcium to plants in culture solution was possible in both neutral and acid media if the content of the culture jars was stirred semi-weekly. In the present experiment the calcium deficiency of the plants in treatment B (Plate 13) indicates that "Versenol" calcium chelate was not a satisfactory carrier for calcium. Whether this failure of chelated calcium to meet the calcium requirement of the plants was due to a relatively lower pH of the solution in treatment B is a question needing further investigation.

Table XI

ROLE OF CALCIUM "VERSENOL" CHELATE
AS A SOURCE OF CALCIUM FOR CORN IN SOLUTION CULTURE

Treatments	Dry weight grams per plant Top	Root	Standard error Top	Root	Visual characteristics
A. Calcium acetate and "Versenol" iron chelate (9 micromoles)	1.14	0.39	.040	.018	Normal and healthy
B. "Versenol" calcium chelate (6 micromoles) and "Versenol" iron chelate (4.5 micromoles)	0.99*	0.36	.055	.025	Involuted leaf margin and brownish root. Drying growing point.
C. "Versenol" iron chelate (9 micromoles) and no calcium	0.74**	0.33*	.030	.015	Involuted leaf margin, dying of youngest leaf; poor root.

*Significantly less (at 0.05 level) than the corresponding value in treatment A.

**Significantly less (at 0.01 level) than the corresponding value in treatment A.

Plate 13

Effect of "Versenol" calcium chelate
on the growth of corn in solution culture.

Ca-Ver is "Versenol" calcium chelate
(6 micromoles)

CONCLUSIONS

Ethyl ammonium phosphate proved to be an effective substitute for potassium dihydrogen phosphate as a source of phosphorus for corn in nutrient solutions. In a nutrient solution of high pH, KH_2PO_4 interacted with ferric chloride and tended to make iron, and possibly phosphorus, unavailable to plants. This problem of iron supply, however, was solved either by using chelated iron (Fe-EDTA) or by substituting ethyl ammonium phosphate for KH_2PO_4.

While ethyl acid phosphate and n-propyl acid phosphate proved to be very poor substitutes for either KH_2PO_4 or ethyl ammonium phosphate, sodium tetra-phosphate took an intermediate position as a source of phosphorus; it was effective on the growth of root but not on the growth of shoot.

Calcium acetate was used successfully as a source of calcium in culture solutions. It not only buffered the solution and kept the pH consistent and high (6.0-7.0) but stimulated the root growth considerably. Under the conditions of the experiments reported here, it is not known, however, whether the action of Ca-acetate on the roots was a direct or an indirect one.

Ethylene diamine tetraacetic acid in low concentrations (10 micromoles and below) was distinctly beneficial to the growth of corn, especially to its root development, in nutrient cultures under greenhouse conditions. This

beneficial effect was attributed to a growth-promoting property of EDTA in low concentrations (45).

The presence of increasing concentrations of copper ions in the nutrient solution causes progressive deterioration in the growth of the plants. Within a certain limit of Cu^{++} concentration, a minute quantity of EDTA (5 micromoles per liter) can completely inactivate this copper-toxicity. Beyond this limit of Cu^{++} concentration, the modifying effect of EDTA on Cu-toxicity appeared to be reduced.

Manganese toxicity, on the other hand, could not be corrected by the addition of EDTA to the nutrient solutions. This was thought to be due to the lack of chelation of Mn^{++} ions by EDTA.

The upper non-toxic level for "Versenol" in a nutrient solution approximated 20 micromoles per liter. Unlike EDTA, "Versenol" did not seem to have any significantly beneficial effect on the growth of corn in solution culture.

The magnesium salt of "Versenol", supplied in non-toxic dilution (6 micromoles per liter) and with proper adjustment of the composition of the solution, substituted successfully for magnesium sulfate as a source of magnesium for corn in solution culture (45).

Plants receiving "Versenol" calcium chelate (6 micromoles per liter) as the only source of calcium began to develop calcium deficiency symptoms to a considerable

degree at the end of the second week of growth.

Suggestions for future work may be made along the following lines: a) to determine how phosphorus from ethyl ammonium phosphate is released and made available to the centers of metabolism within the plant; b) to determine the physiological basis for the beneficial effect of EDTA at low concentration on the growth of plants in solution culture (e. g. respiration or enzyme studies); c) to employ other synthetic chelating agents and to evaluate their use in solution culture; d) to determine the use of "Versenol" magnesium chelate for plants susceptible to magnesium deficiency (such as apple) in solution culture.

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